
A CONTRIBUTION TO THE STUDY OF POLLEN WALL ULTRASTRUCTURE OF ORCHID POLLINIA¹

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ABSTRACT

Pollen grains of 30 orchid species from the Spiranthoideae and Epidendroideae (including the Vandaeae) were studied ultrastructurally. This study complements the published palynological literature on the Apostasioideae, Cyripedioideae, Spiranthoideae, and Orchidoideae. The orchid pollen thus far studied exhibits a wide range of variation in pollen unit, aperture type, and wall ultrastructure. The least specialized Apostasioideae and some Cyripedioideae shed pollen in monads and have monosulcate grains with a tectate-columellate, perforate exine structure. These are features observed in most primitive monocotyledons. In the more specialized orchid subfamilies, pollen occurs in mealy or tightly packed, waxy pollinia, and the grains have a porate-ulcerate aperture or are inaperturate, and the sporopollenin wall is present only on the most peripheral grains in the pollinium (a few species lack exines). The presence of the exine on only the most peripheral grains in the pollinium is accompanied by a loss of the footlayer in many taxa and elaboration of the intine. As in other monocots, endexine has not been unequivocally demonstrated in orchids.

The Orchidaceae comprise about 1,000 genera and 15,000–20,000 species. Dressler (1981, 1983) has separated the orchids into five subfamilies: Apostasioideae, Cyripedioideae, Spiranthoideae, Orchidoideae, and Epidendroideae (including the Vandaeae).

There is general agreement among taxonomists that the Apostasioideae are the least specialized (primitive) subfamily of the Orchidaceae (Dressler & Dodson, 1960; Dressler, 1981, 1983), with two genera and about 16 species, found primarily in tropical Asia. Pollen morphology and ultrastructure have been investigated (Schill, 1978; Newton & Williams, 1978) and the pollen shows features of many other monocots (Zavada, 1983). The grains of the Apostasioideae are usually shed in monads and are monosulcate. Exine sculpturing is reticulate. Pollen wall structure is tectate-columellate with a thin footlayer and no endexine.

The Cyripedioideae, primarily a tropical group, have four genera and about 130 species. This subfamily likewise exhibits many primitive features (Ackerman & Williams, 1980, 1981). Pollen is generally monosulcate or ulcerate (i.e., with an ill-defined pore). Exine sculpturing can be reticulate or verrucate to scabrate. The pollen wall infrastructure, as revealed by SEM, appears to be columellate (Newton & Williams, 1978; Schill & Pfeiffer, 1977).

Representative taxa of the rather large subfam-

ily Spiranthoideae have been investigated palynologically to some extent (Balogh, 1979; Williams & Broome, 1976). Pollen grains are shed in loose pollinia and are generally inaperturate. Exine sculpturing is reticulate (especially in the Spiranthinae), and wall structure varies depending on the position of the pollen grain in the pollinium: peripheral grains are usually tectate-columellate with a thick footlayer; those in the interior of the pollinium usually lack the tectum, with only the columellae and footlayer being present.

Pollen of the Spiranthoideae and Epidendroideae have been little studied using transmission electron microscopy (however, see examples in Chardard, 1958, 1969; Heslop-Harrison, 1968; Dulieu 1973; Caspers & Caspers, 1976; Balogh, 1982; Balogh & Mann, 1982; Hesse & Burns-Balogh, 1984; Wolter & Schill, 1986). The present contribution describes the pollen wall structure of 30 taxa from these subfamilies (only one taxon from the Spiranthoideae).

METHODS

Pollinia were removed from living material and placed immediately in cacodylate HCl-buffered glutaraldehyde-formaldehyde, followed by fixation in osmium tetroxide, dehydration in an ethanol series, and embedding in Dow Epoxy Resin 334. Pollinia were sectioned on an LKB-1 ultramicrotome, se-

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quentially poststained in uranyl acetate and lead citrate for 15 minutes, and viewed with a Philips EM-300 transmission electron microscope. Vouchers for all live specimens have been deposited in the herbaria CONN or MRD (Appendix 1). The classification used follows Dressler (1981, 1983).

DESCRIPTIVE PALYNOLOGY

SPIRANTHOIDEAE

Erythrodeae-Spiranthinae

Sacoila lanceolata (Aubl.) Garay. Pollen grains occur in loosely packed pollinia and are inaperturate. Exine sculpturing is reticulate and pollen wall structure varies depending on the position of the pollen grain in the pollinium. Pollen grains peripheral in the pollinium are tectate-columellate with a relatively thick footlayer (Fig. 1). Pollen in the interior of the pollinium usually lack a tectum;

however, the columellae and footlayer are present (Fig. 2). The footlayer is underlain by a thin fibrillar intine (Fig. 2).

EPIDENDROIDEAE

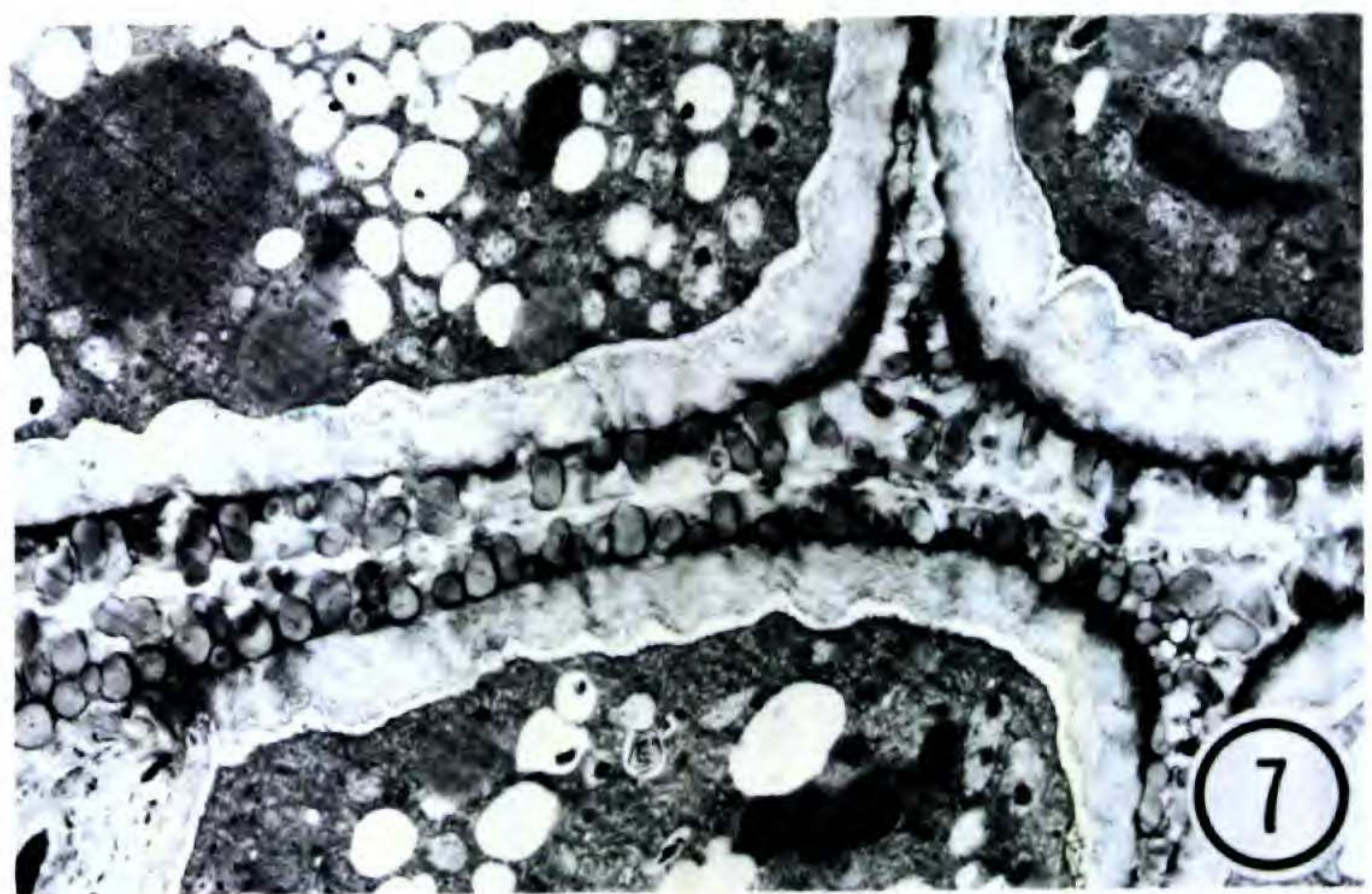
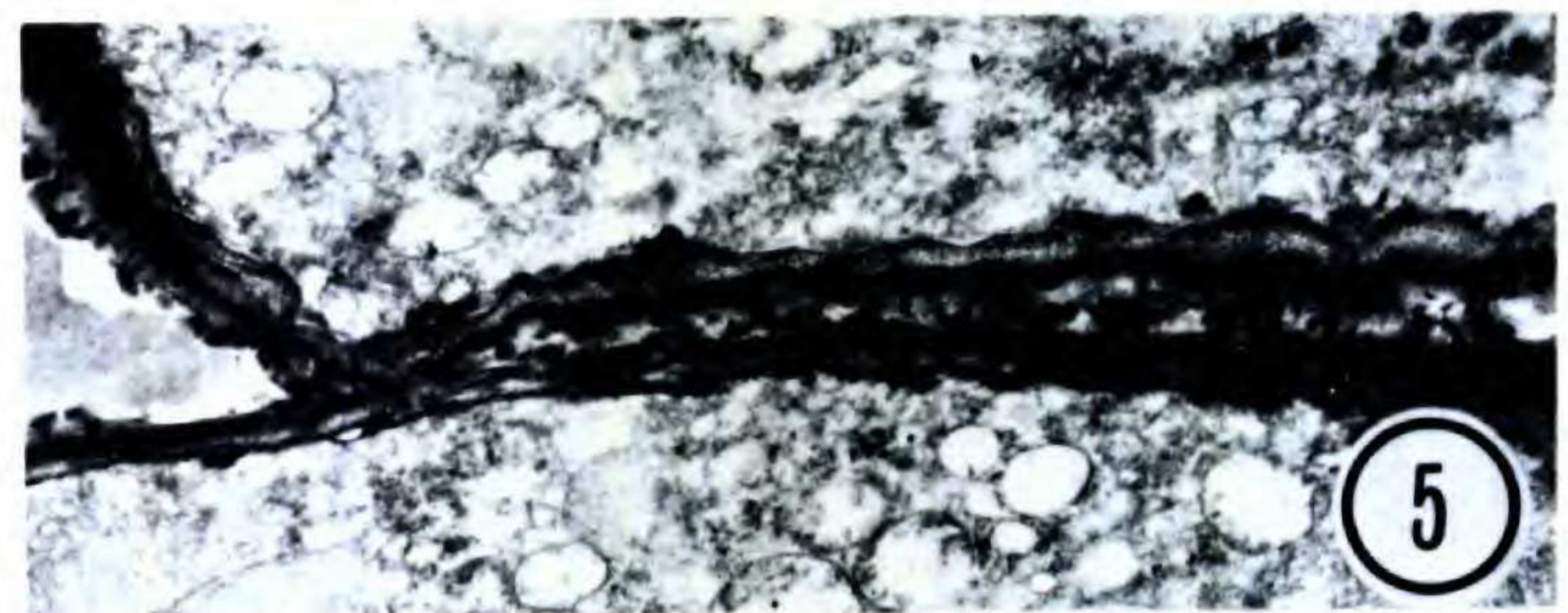
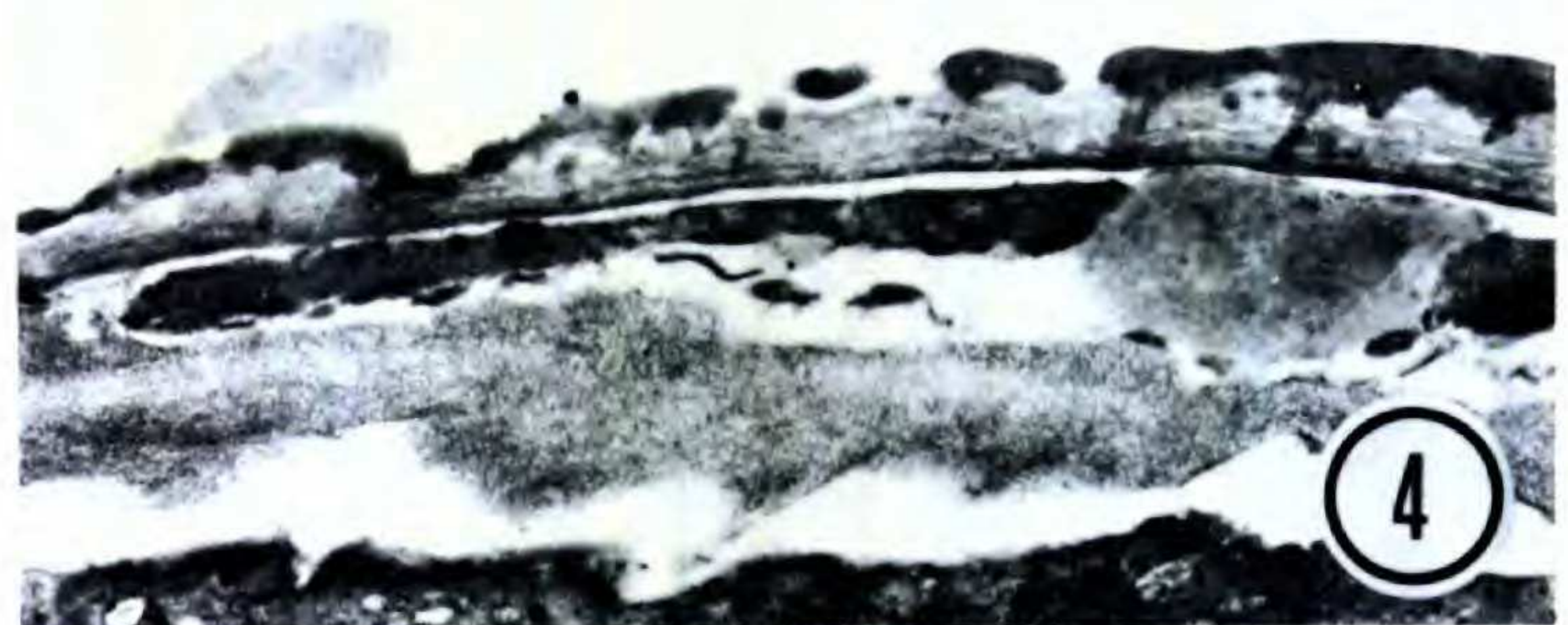
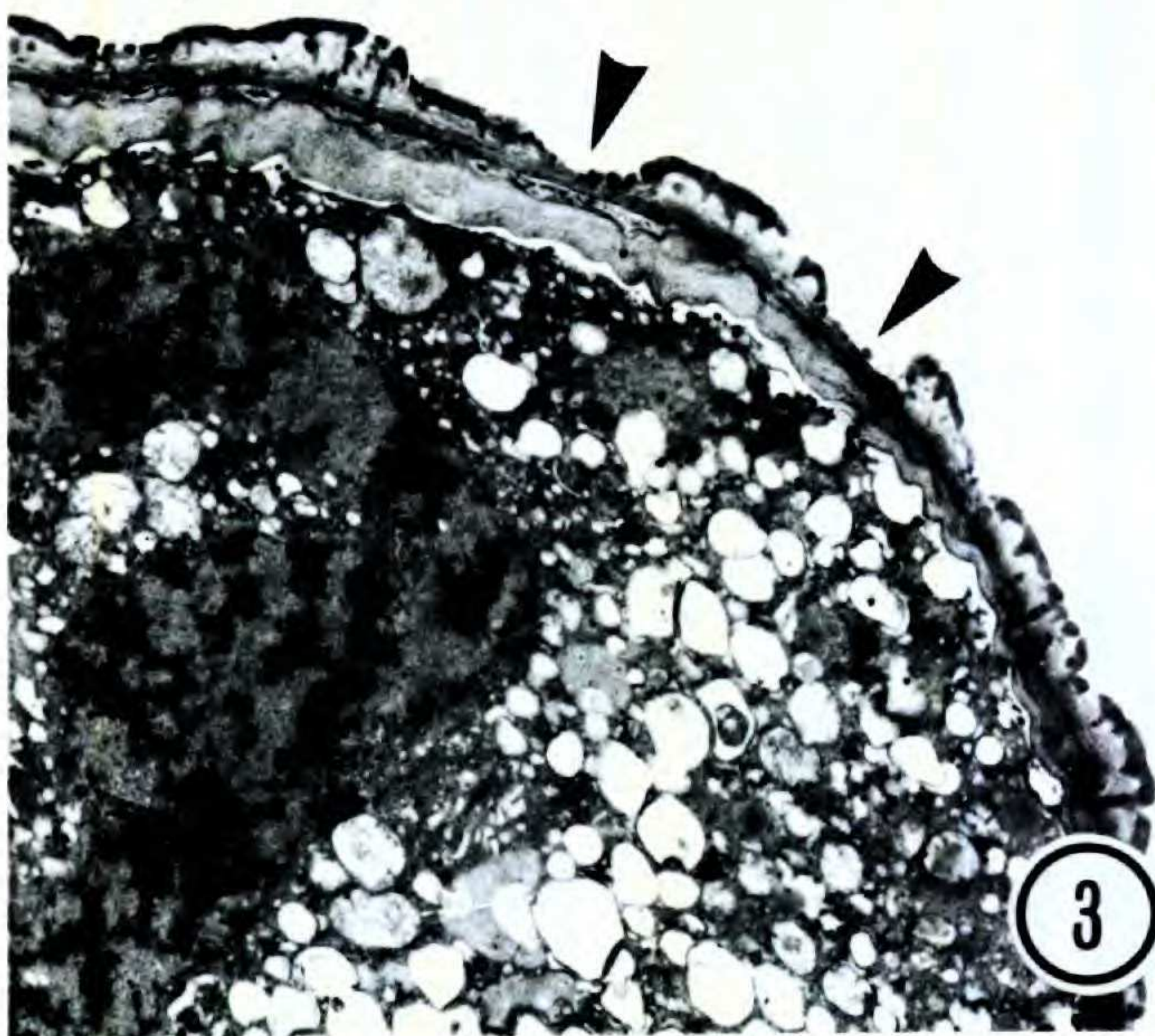
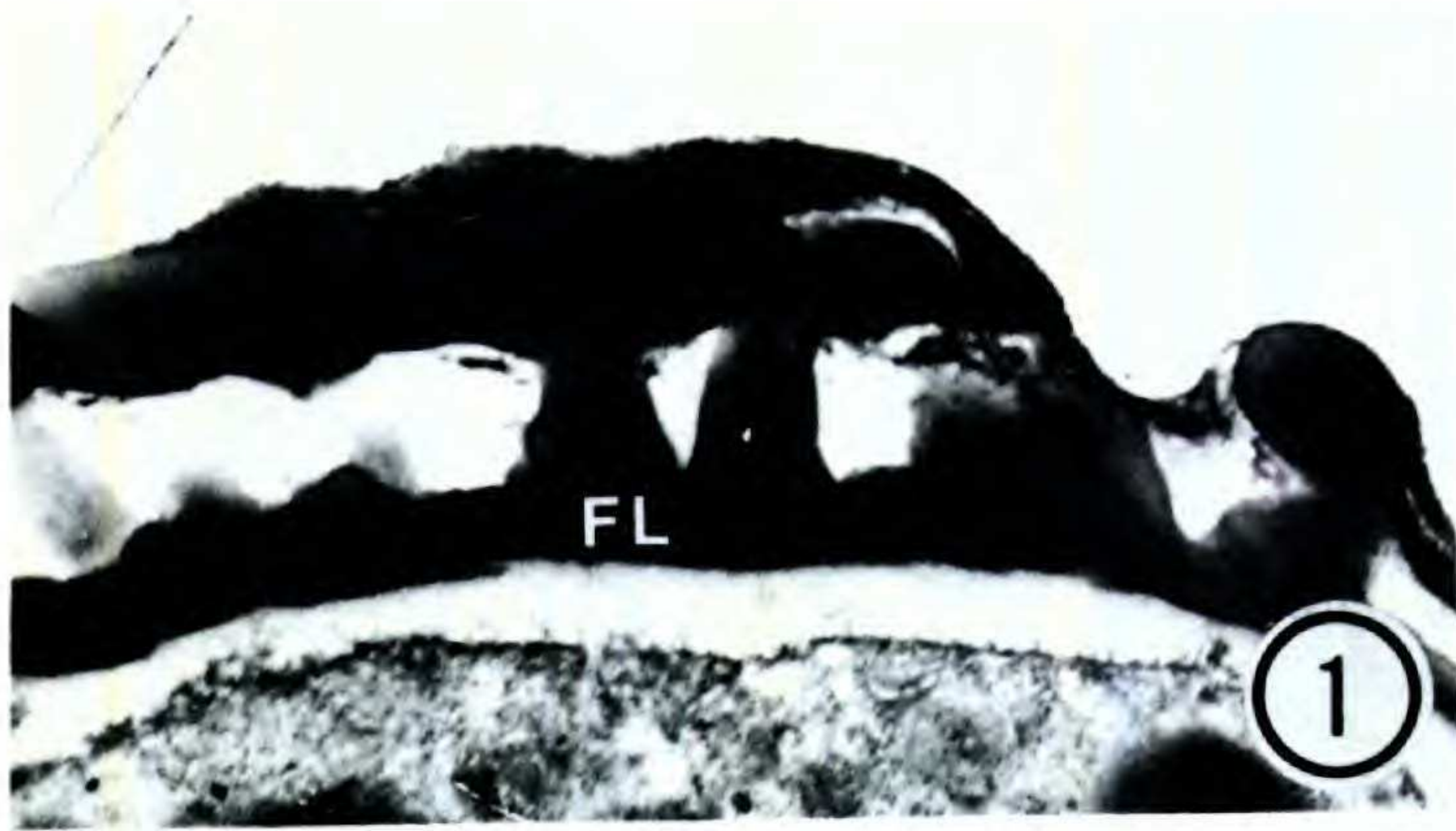
Arethuseae-Bletiinae

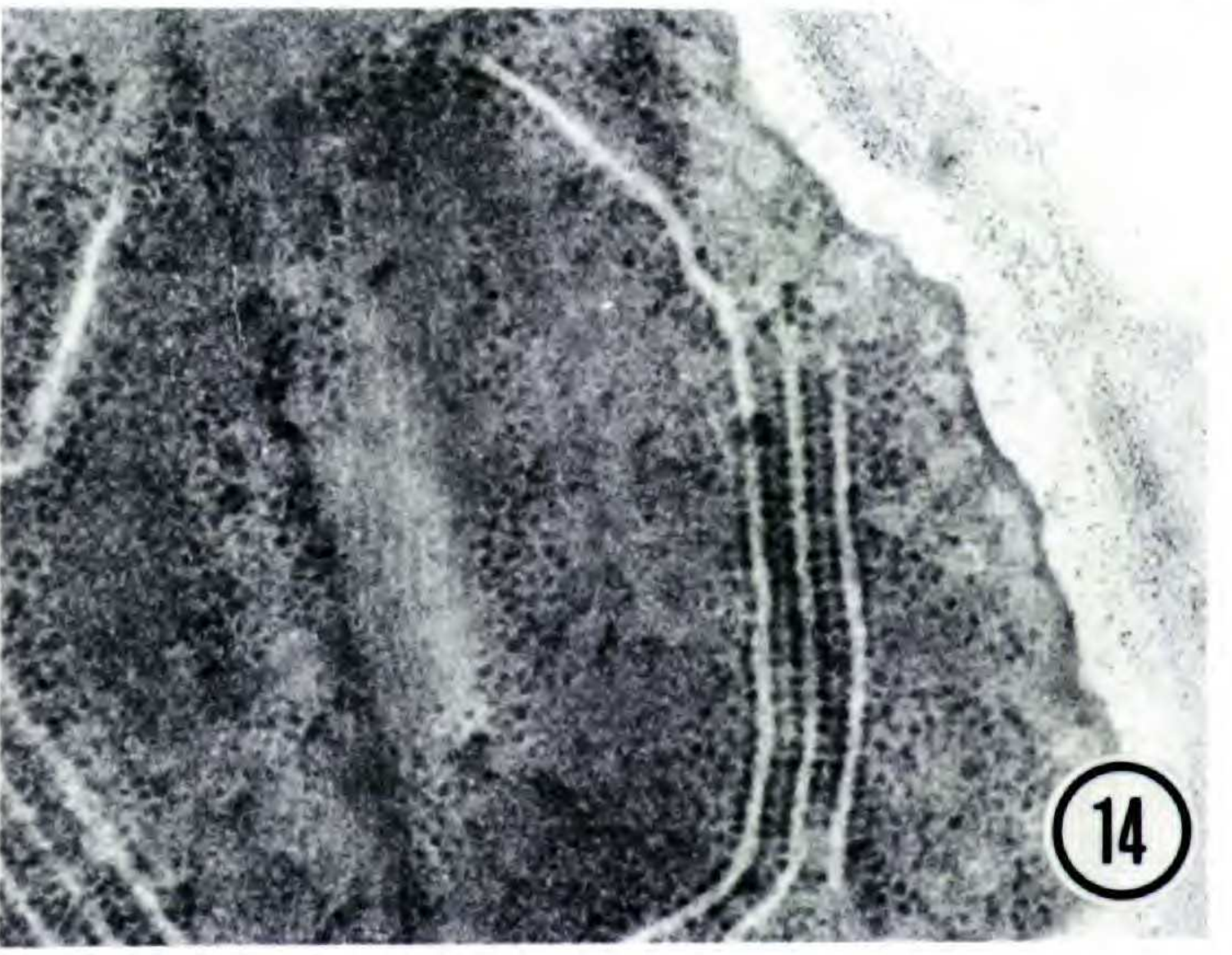
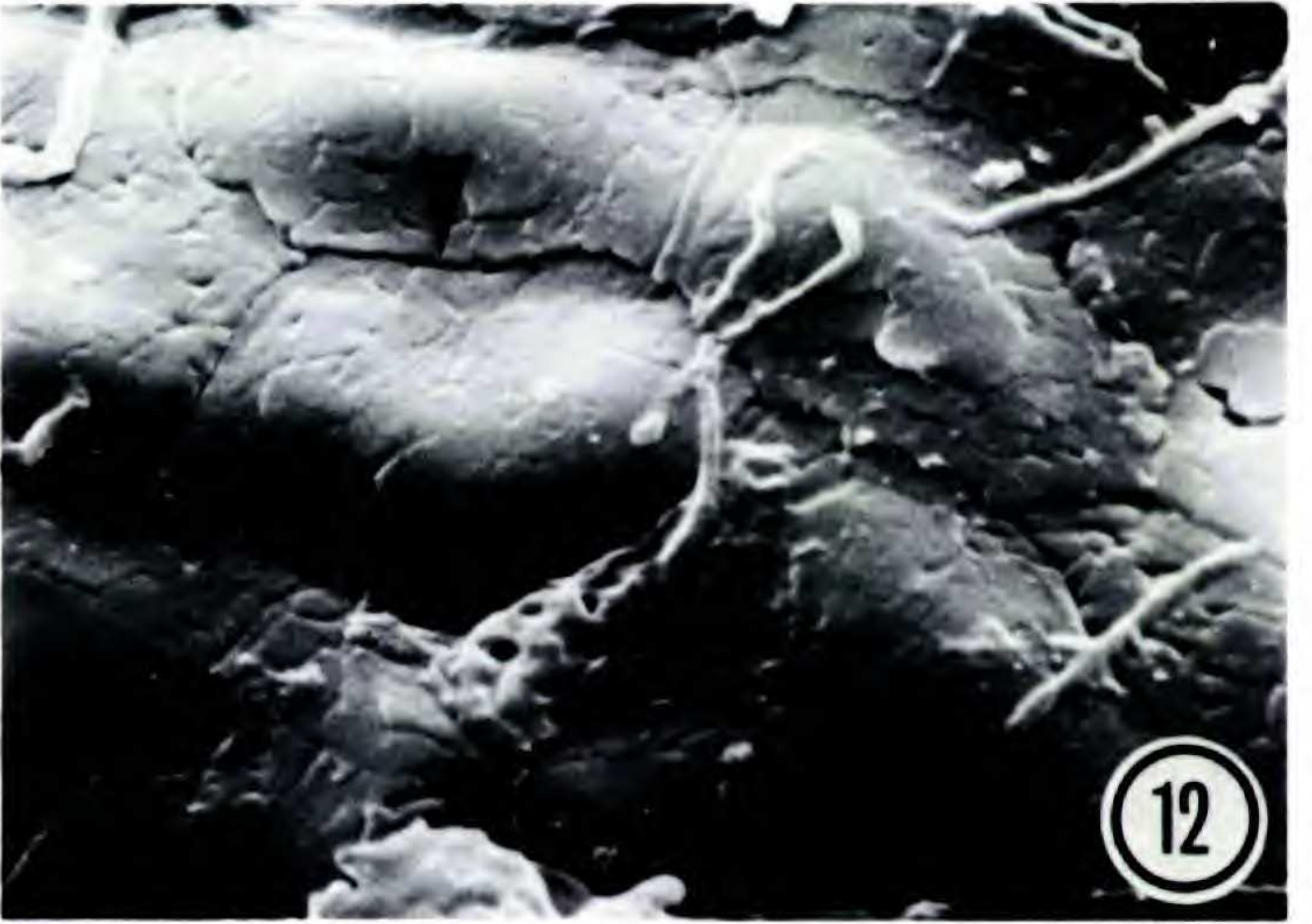
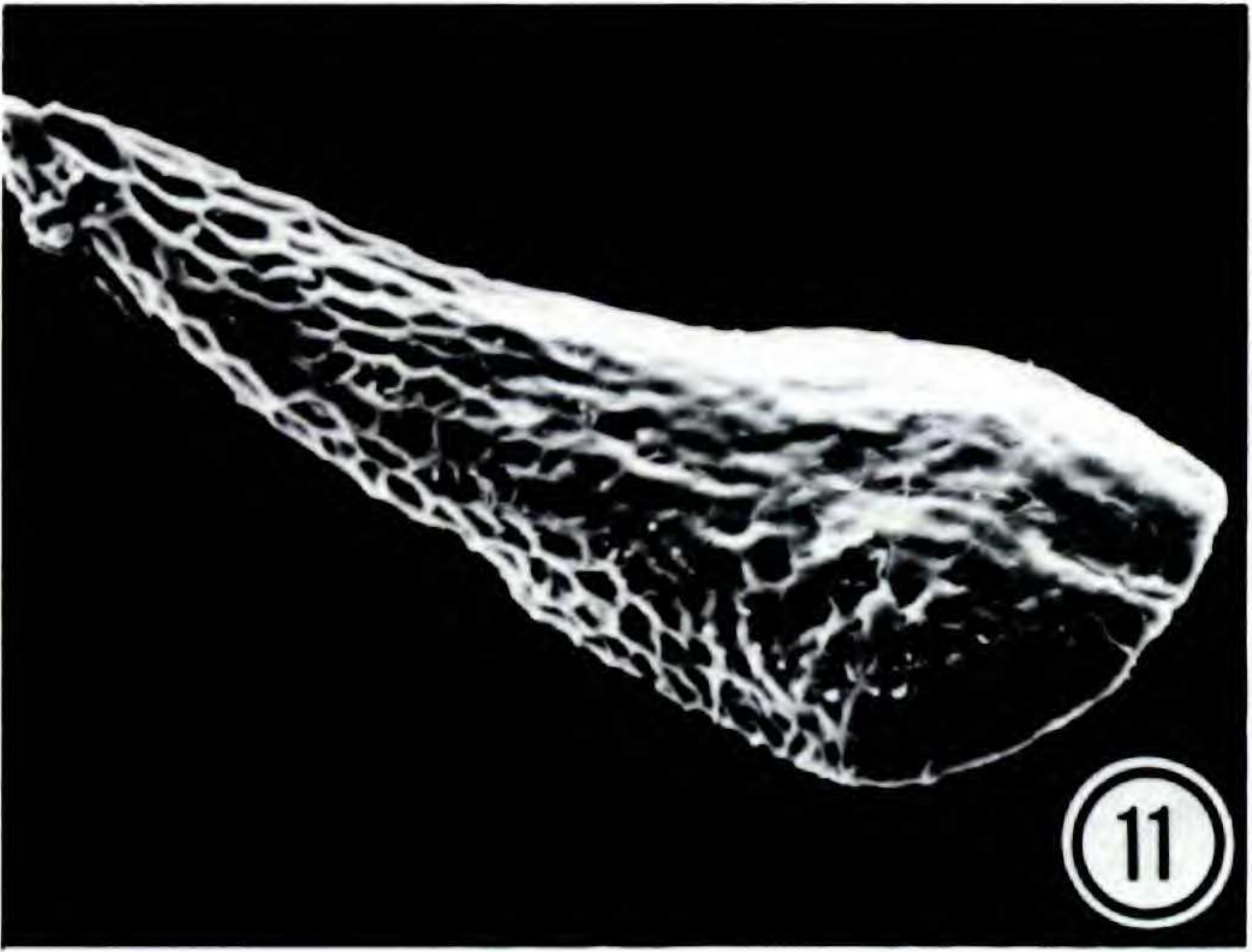
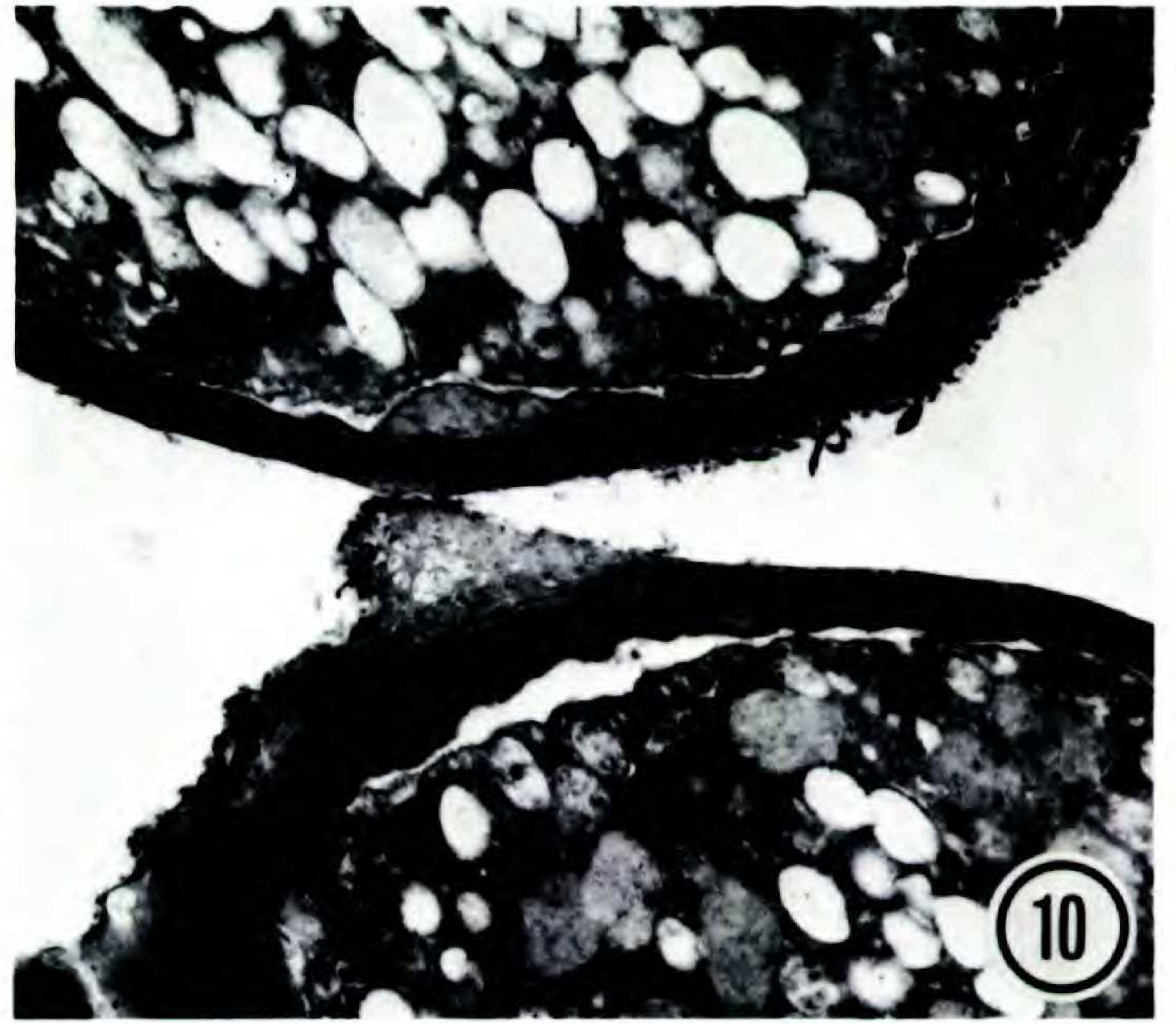
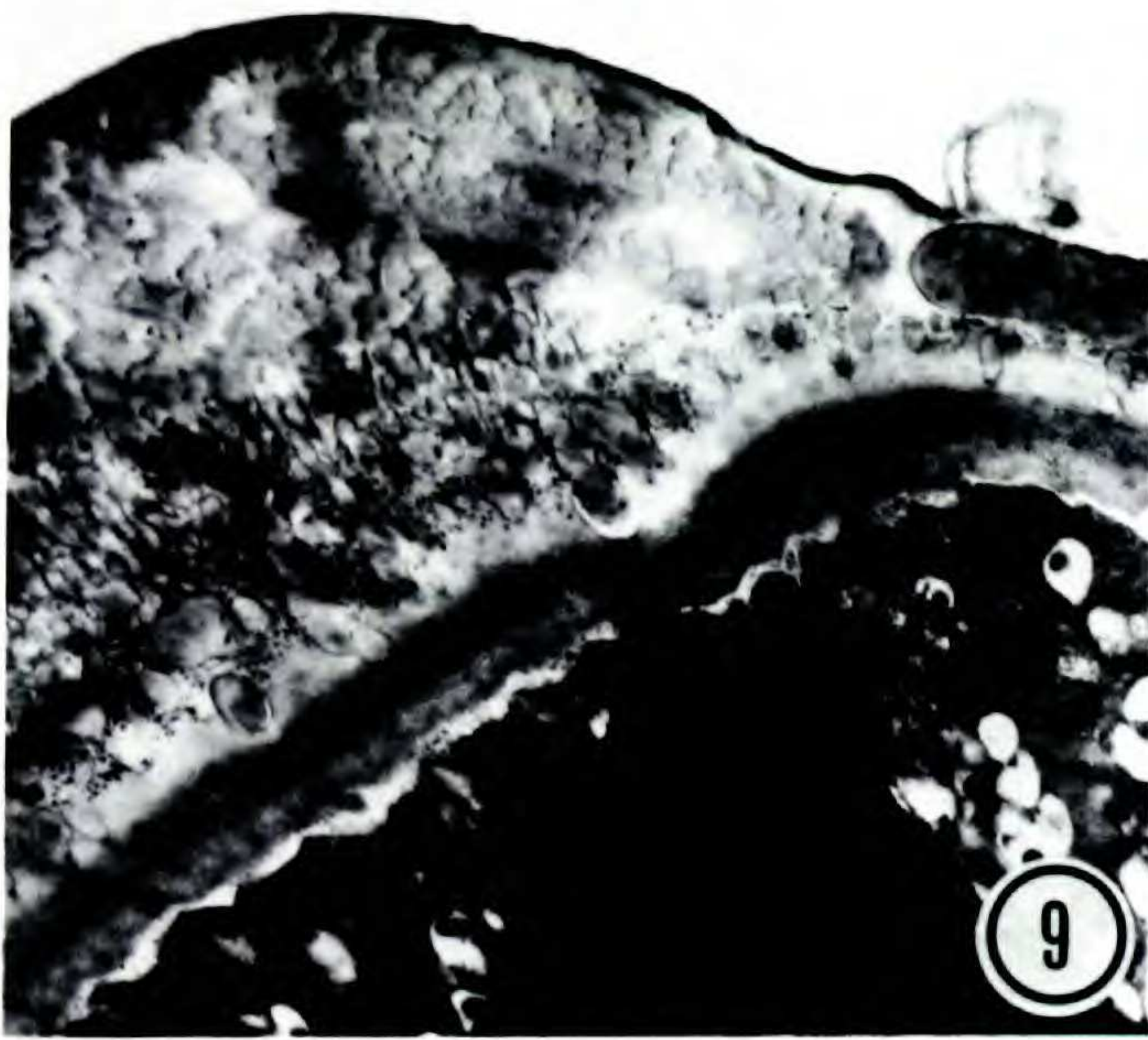
Acanthephippium sylhetense Lindl. and *Bletilla striata* (Thunb.) Reichb. f. In both species pollen occurs in tightly packed pollinia. Pollen of *Bletilla striata* appears multiaperturate in the peripheral region of the pollinium, the exine having a number of thin areas (Fig. 3). Pollen wall structure is tectate-columellate (Fig. 4). The columellae are thin and rest on a unilamellar footlayer that is underlain by a thick multilayered intine (Fig. 4). Pollen in the interior of the pollinium lack an exine and are surrounded by intine; however, there appear to be small sporopollenin granules embedded in the outer portion of the intine (Fig. 5).

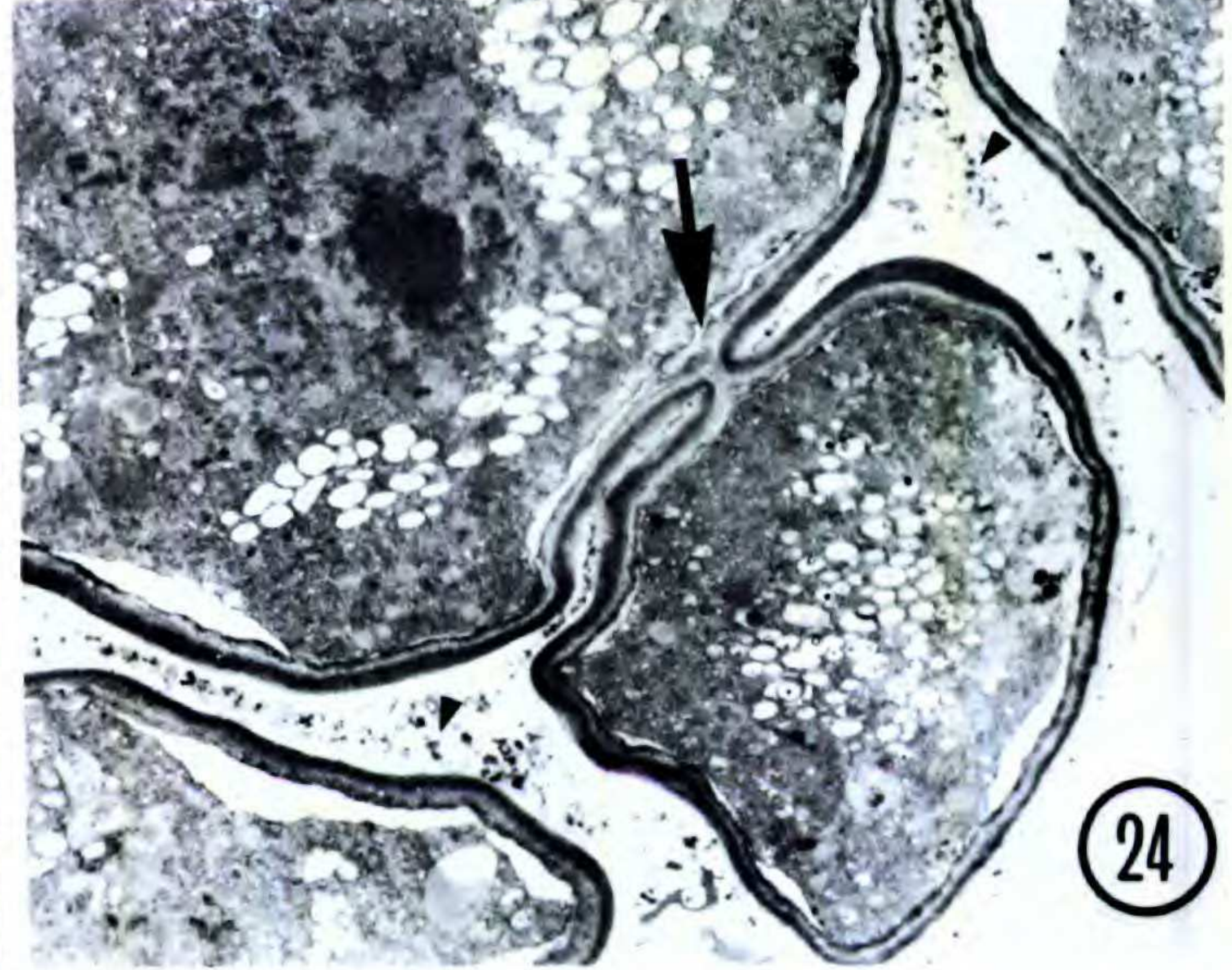
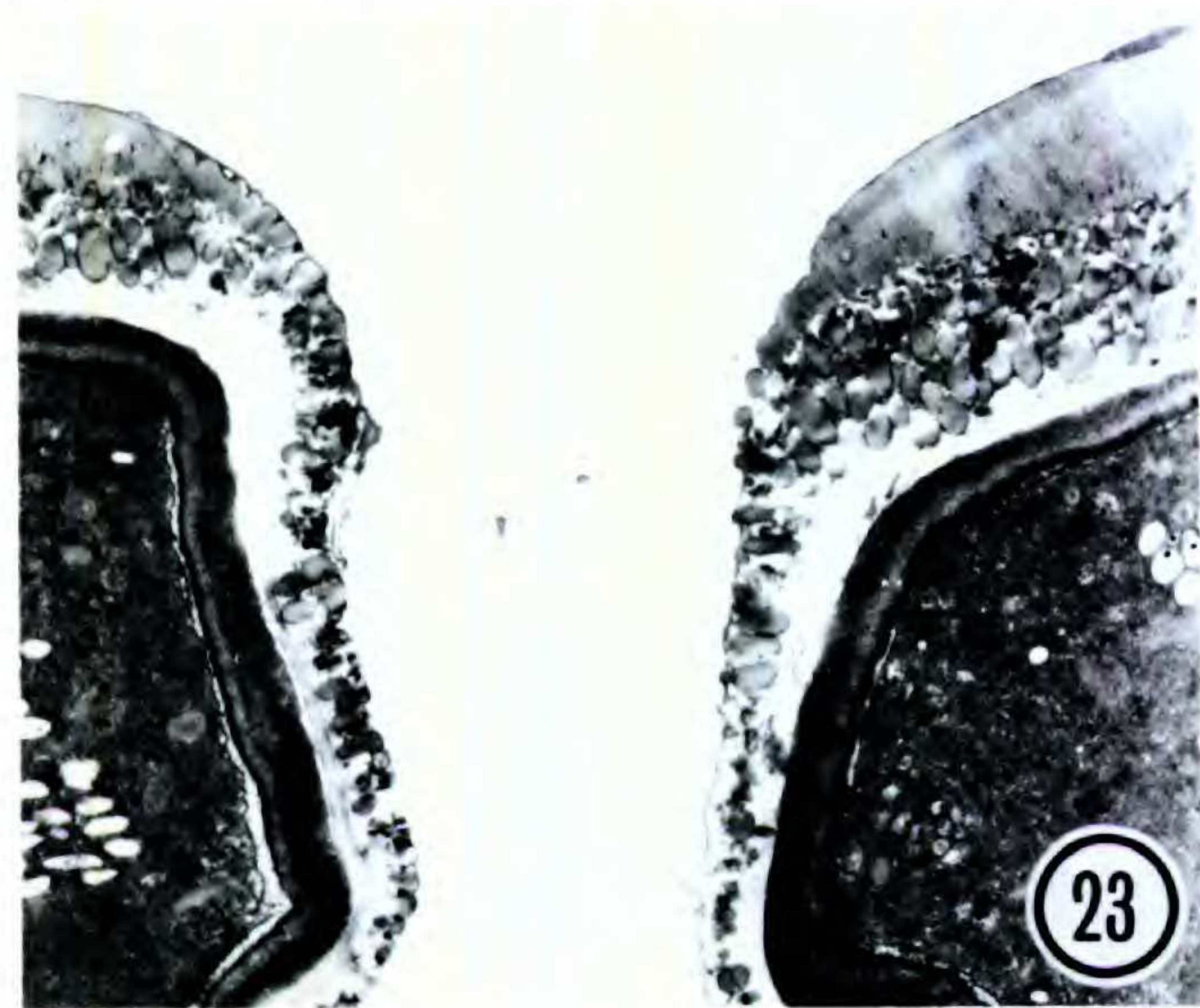
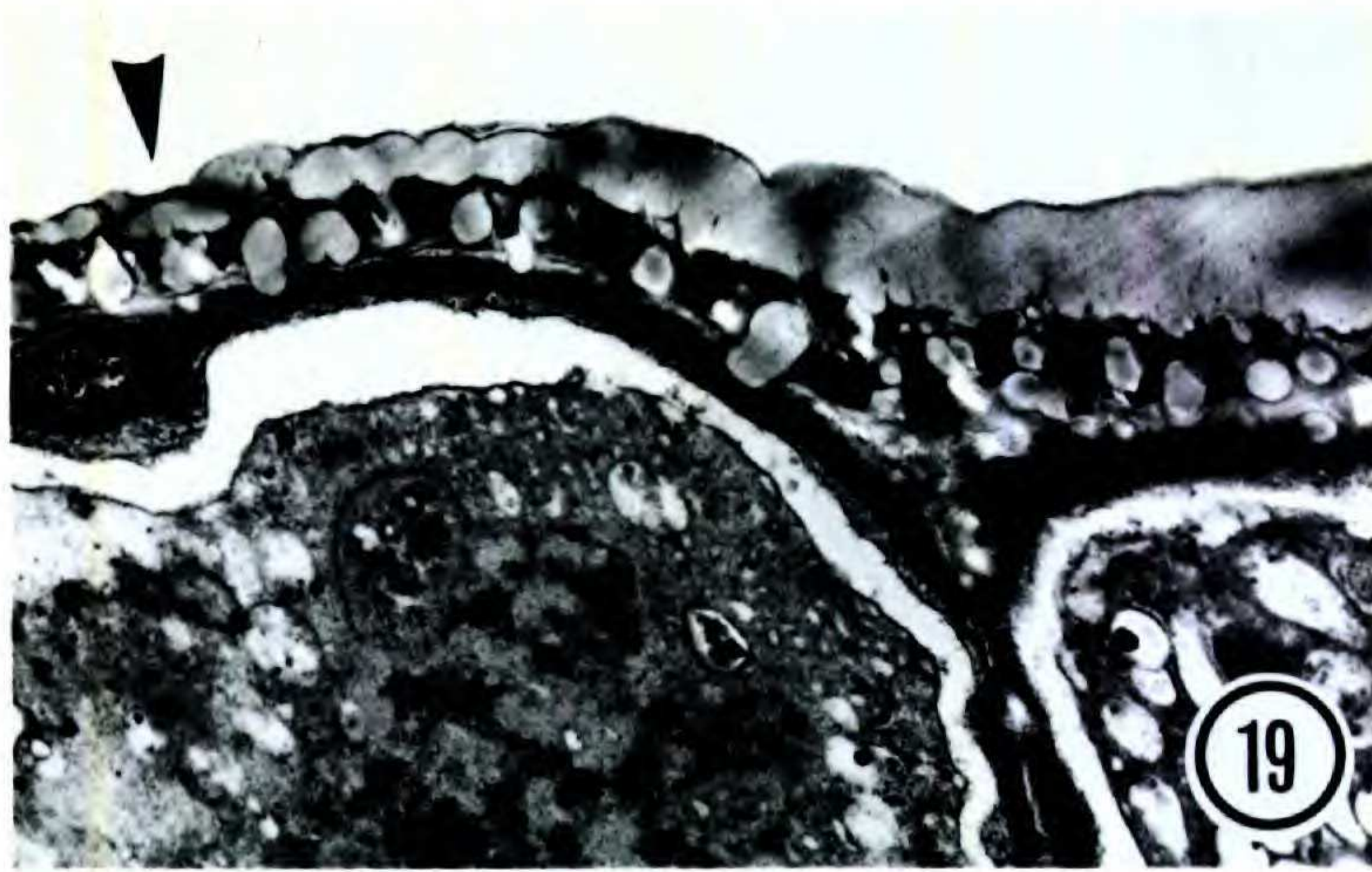
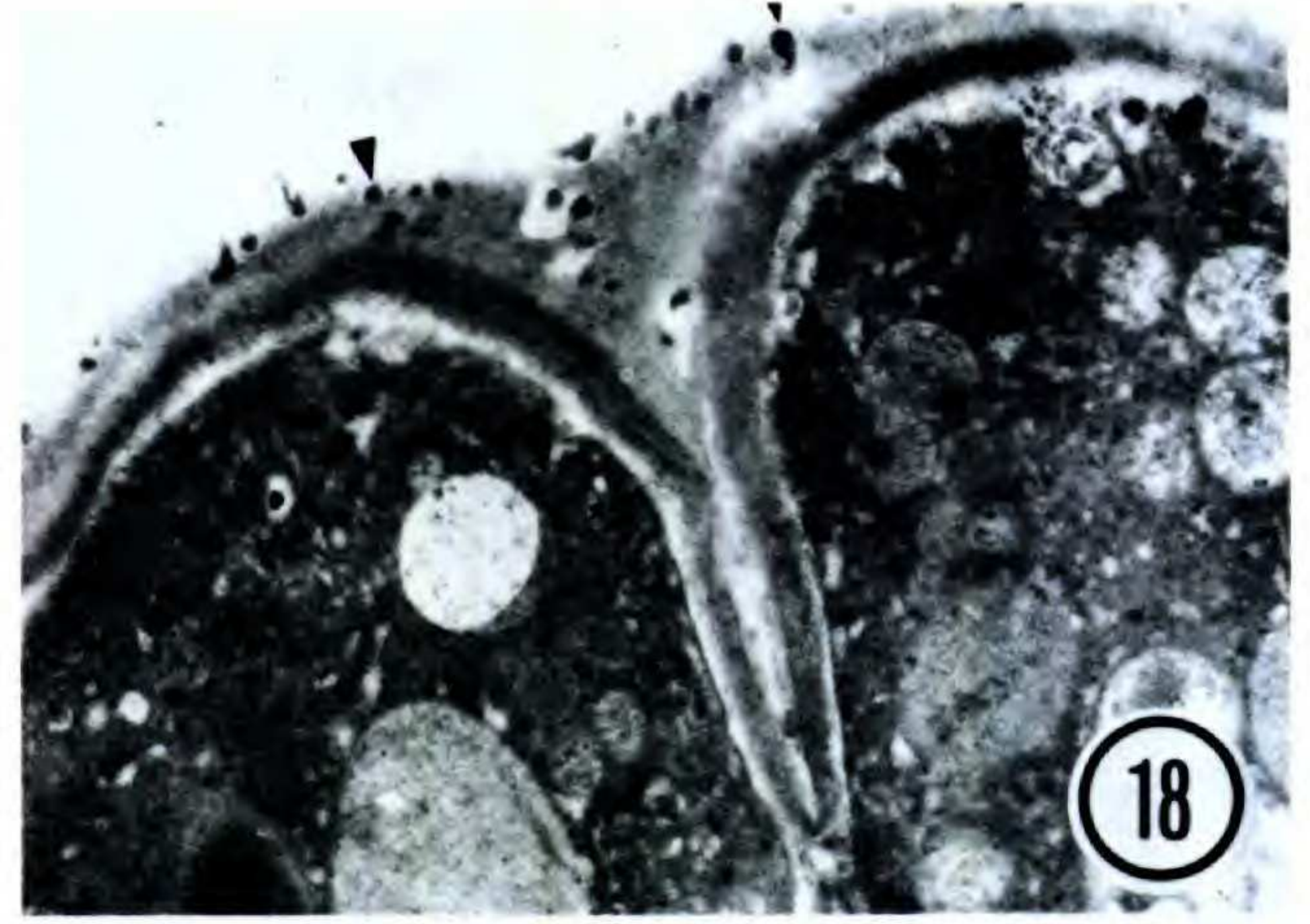
FIGURES 1-8. 1, 2. Pollen of the Spiranthoideae.—1. *Sacoila lanceolata*, pollen peripheral in the pollinium showing tectate-columellate wall structure with the well-developed footlayer (FL), $\times 11,500$.—2. *S. lanceolata* pollen interior in the pollinium showing columellae, footlayer, and the thin fibrillar intine. The tectum appears to be absent, $\times 6,500$. 3-8. Pollen of the Epidendroideae.—3. *Bletilla striata* pollen peripheral in the pollinium showing tectate-columellate wall, the thin footlayer, and the thin areas of the exine (arrowheads) that may function as apertures, $\times 6,500$.—4. High magnification of *B. striata* pollen peripheral in the pollinium showing wall structure, $\times 8,200$.—5. Pollen of *B. striata* more interior in the pollinium showing absence of exine, $\times 8,200$.—6. *Acanthephippium sylhetense* pollen peripheral in the pollinium showing tectum, infrastructural layer of oval columellae-like granules that rest on a thin intine. Note the dense nonsporopollenin substance that coats the entire pollinium (arrowhead), $\times 3,450$.—7. *A. sylhetense* pollen near the periphery of the pollinium showing oval columellae-like granules that rest on the intine (tectum is absent), $\times 5,300$.—8. *A. sylhetense* pollen most interior in the pollinium showing the fibrillar intine. No sporopollenin wall is present, $\times 8,200$.

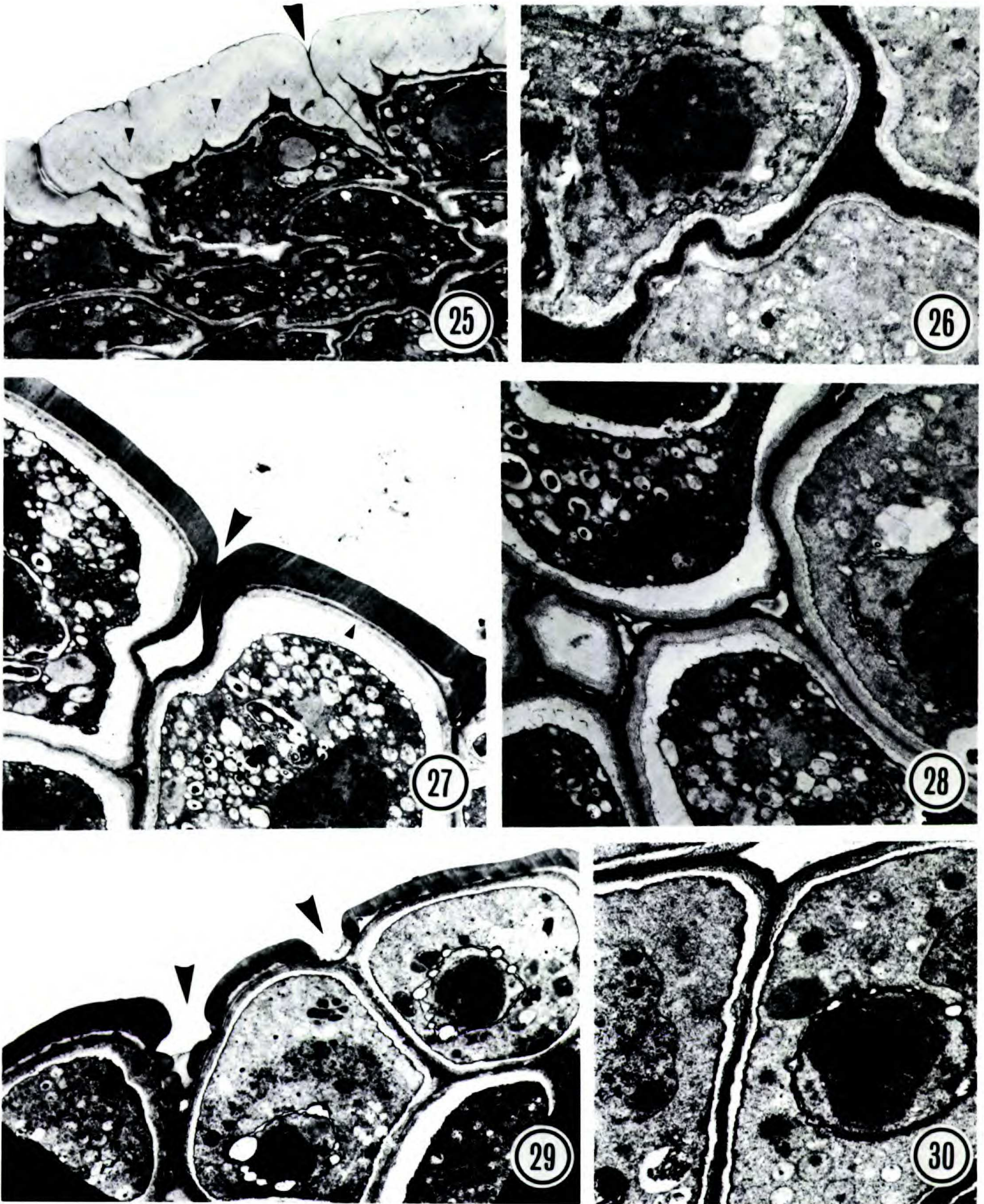
FIGURES 9-16. Pollen of the Epidendroideae.—9. *Coelogyne nitida* pollen peripheral in the pollinium showing the thick tectum and granular infrastructural layer that rests directly on the intine, $\times 6,500$.—10. *C. nitida* pollen interior in the pollinium showing multilayered fibrillar intine. Exine is absent, $\times 6,500$.—11. *Malaxis paludosa* pollinium, $\times 200$.—12. High magnification of *M. paludosa* pollinium showing sporopollenin outer wall. Note the "sutures" between individual pollen grains (arrowheads), $\times 2,000$.—13. *M. latifolia* pollen peripheral in the pollinium. Note suture between pollen grains (arrowhead, compare to the surface features of *M. paludosa*, Fig. 12), $\times 3,030$.—14. *M. latifolia*, cytoplasm of pollen interior in the pollinium showing the condensed rough endoplasmic reticulum, a structure often found in resting cells of animals, but rare in plants, $\times 37,770$.—15. *M. paludosa* pollen peripheral in the pollinium showing atectate wall, $\times 7,000$.—16. *M. unifolia*, pollen peripheral in the pollinium showing atectate wall, $\times 7,000$.

FIGURES 17-24. Pollen of the Epidendroideae.—17. Pollen peripheral in the pollinium of *Cattleya skinneri* showing oval columellae-like structures that rest on a thin intine, $\times 15,125$.—18. Pollen interior in the pollinium of *C. skinneri* showing exineless pollen. Note small sporopollenin granules embedded in the outer layer of the intine (arrowheads), $\times 12,075$.—19. Pollen peripheral in the pollinium of *Encyclia cochleata* showing the thick tectum, and the granular layer that rests directly on the intine. Note the thinning of the tectum (arrowhead) that may act as an aperture, $\times 20,500$.—20. Pollen from the interior of the pollinium of *E. cochleata* showing fibrillar intine; often sporopollenin granules (arrows) are embedded in this wall layer at the juncture of three or more pollen grains, $\times 6,500$.—21. Pollen peripheral in the pollinium of *Epidendrum anceps* showing the tectum and the granular layer that rests directly on the thick intine. Note the thinning of the tectum (arrowhead), $\times 11,200$.—22. Pollen interior in the pollinium of *E. anceps* showing exineless pollen. Note the minute sporopollenin granules between pollen grains, $\times 4,150$.—23. *Laelia autumnalis* pollen peripheral in the pollinium showing the tectum on the outermost wall and the granular infrastructural layer that rests on a multilayered intine, $\times 4,800$.—24. Pollen interior in the pollinium of *L. autumnalis* showing exineless pollen with a multilayered intine. Note cytoplasmic connection between adjacent pollen grains (arrow) and minute granules (arrowheads), $\times 3,500$.









FIGURES 25-30. Pollen of the Epidendroideae.—25. Pollen peripheral in the pollinium of *Masdevallia caudata* showing thick atectate exine that is underlain by a thin intine. Note the microchannels that traverse the lower portion of the exine (small arrowheads) and the “suture” between adjacent pollen grains (large arrowhead), $\times 5,300$.—26. Pollen interior in the pollinium of *M. caudata* showing exineless pollen grains that are surrounded by a thin intine. The dense nonacetolysis resistant layer between pollen grains may be part of the intine, $\times 10,000$.—27. Pollen peripheral in the pollinium of *Dryadella edwallii* showing atectate exine with thin bilayered intine (small arrowhead). Note suture between adjacent pollen grains. When the tectum of adjacent pollen grains is closely appressed to one another the exine may prevent dessication of the exineless interior pollen grains. As the pollinium expands these sutures may open permitting the emergence of the pollen tubes (also see Figs. 15, 16, 25, 29), $\times 6,500$.—28. Pollen interior in the pollinium of *D. edwallii* showing multilayered intine, $\times 10,000$.—29. Pollen peripheral in the pollinium of *Pleurothallis janetiae* showing atectate exine. Note the suture between adjacent pollen grains (arrowheads), $\times 5,800$.—30. Pollen interior in the pollinium of *P. janetiae* showing the presence of only the intine, $\times 10,500$.

Acanthephippium sylhetense pollen grains are inaperturate. Exine sculpturing is psilate. The wall structure of pollen peripheral in the pollinium is tectate with oval columellae-like elements in the infrastructure (Fig. 6). The closely spaced columellae-like elements are often thick branching rods to oval and granular-like structures that support the tectum (Fig. 6). The bases of the columellae rest directly on a thin fibrillar intine. In the interior of the pollinium the tectum is generally absent, being comprised of short, stout columellae or irregularly shaped granules (Fig. 7). The exine is entirely absent from the innermost pollen in the pollinia; each pollen grain is surrounded by a fibrillar intine (Fig. 8).

Coelogyneae-Coelogyneinae

Coelogyne nitida Lindl. Pollen occurs in tightly packed pollinia and appears inaperturate. Pollen peripheral in the pollinium have a thick tectum, which is underlain by a massive granular layer (Fig. 9). The granular layer rests directly on a thick bilayered intine (Fig. 9). Pollen interior in the pollinium lack an exine, and each pollen grain is surrounded by a bilayered intine (Fig. 10).

Malaxideae

Malaxis latifolia J. E. Sm., *M. paludosa* Sw., and *M. unifolia* Michx. Pollen occurs in small, tightly packed pollinia and are inaperturate (Figs. 11, 12). The outer wall of pollen peripheral in the pollinium is thick and homogeneous (atectate) (Figs. 13, 15, 16). Each pollen grain peripheral in the pollinium has its own wall (Figs. 12, 13); however, the walls are closely appressed to one another, often giving the appearance that the entire pollinium is enclosed by a single sporopollenin wall. Pollen interior to these outermost pollen grains appear to lack exine in *M. latifolia* (Fig. 13) and are surrounded only by intine; however, a thin homogeneous sporopollenin wall surrounds each microspore in the two North American species (Figs. 15, 16). All three species investigated are identical with regard to wall ultrastructure of pollen most peripheral in the pollinium. The cytoplasm in *M. latifolia* has what appears to be condensed rough endoplasmic reticulum (Fig. 14), a condition often found in resting cells in animals, but a rare occurrence in plants.

Epidendreae-Laeliinae

Cattleya skinneri Batem., *Encyclia cochleata* (L.) Lemee, *Epidendrum anceps* Jacq., and *Laelia*

autumnalis Lindl. Pollen in all four taxa occur in tightly packed pollinia. Pollen grains peripheral in the pollinium have a thick tectum underlain by large, stout columellae or oval granules that rest directly on the intine (Figs. 17, 19, 21). Pollen more interior in the pollinium are exineless and surrounded only by an intine (Figs. 18, 20, 22). However, at the junctures of two or more pollen grains, sporopollenin granules are evident (*Cattleya*, Fig. 18 and *Encyclia*, Fig. 20). In *Epidendrum* (Fig. 22) minute sporopollenin granules are embedded in the intine between adjacent pollen grains.

Laelia autumnalis deviates from the above taxa by the occurrence of the tectate-granular wall. Pollen grains peripheral in the pollinium have a thick tectum underlain by a granular layer (Fig. 23). The tectum tapers out on the lateral faces of the peripheral pollen grains (Fig. 23). Pollen in the interior of the pollinium are similar to *Epidendrum* in having minute sporopollenin granules embedded in the outer layer of the intine (Fig. 24).

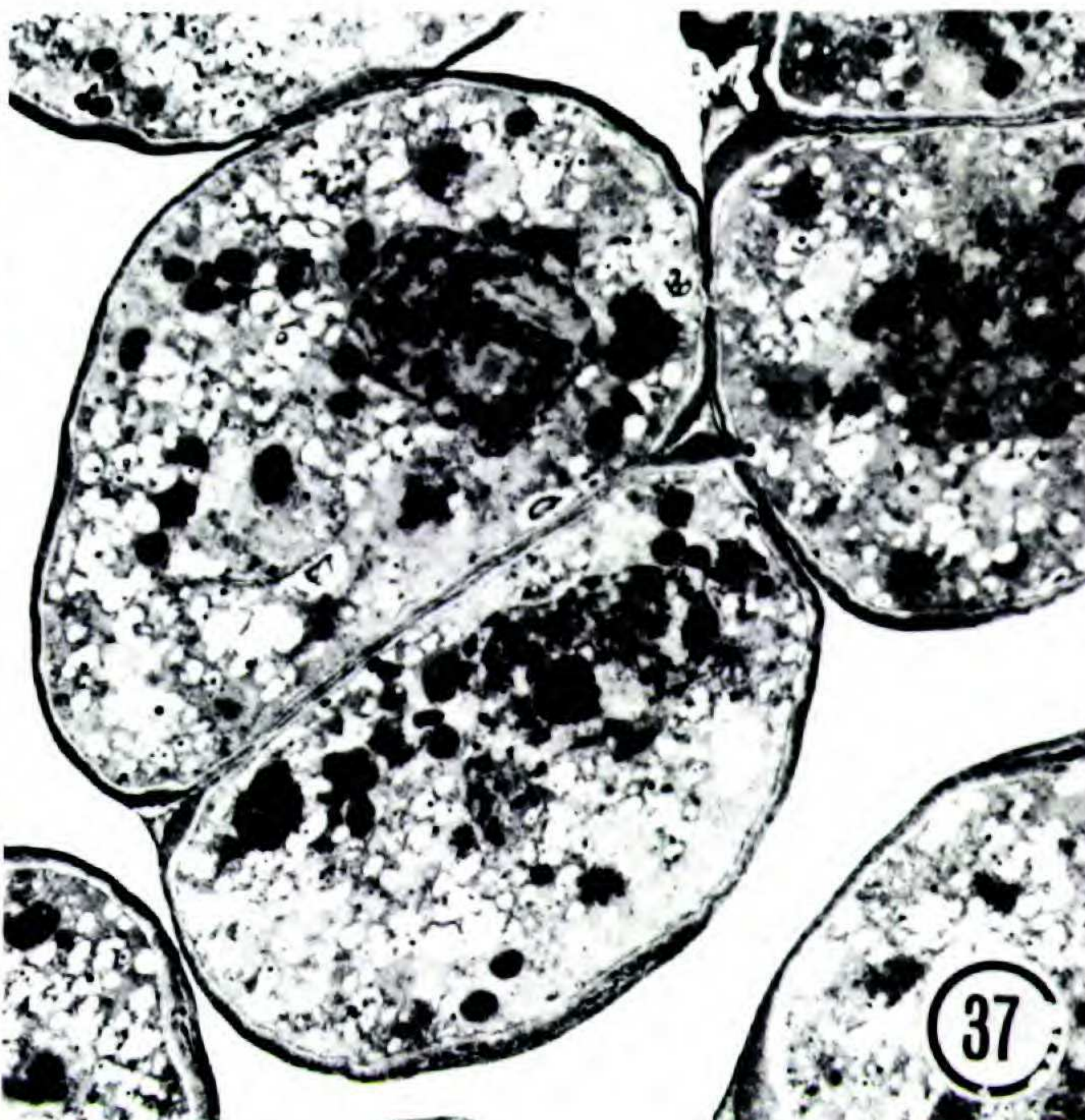
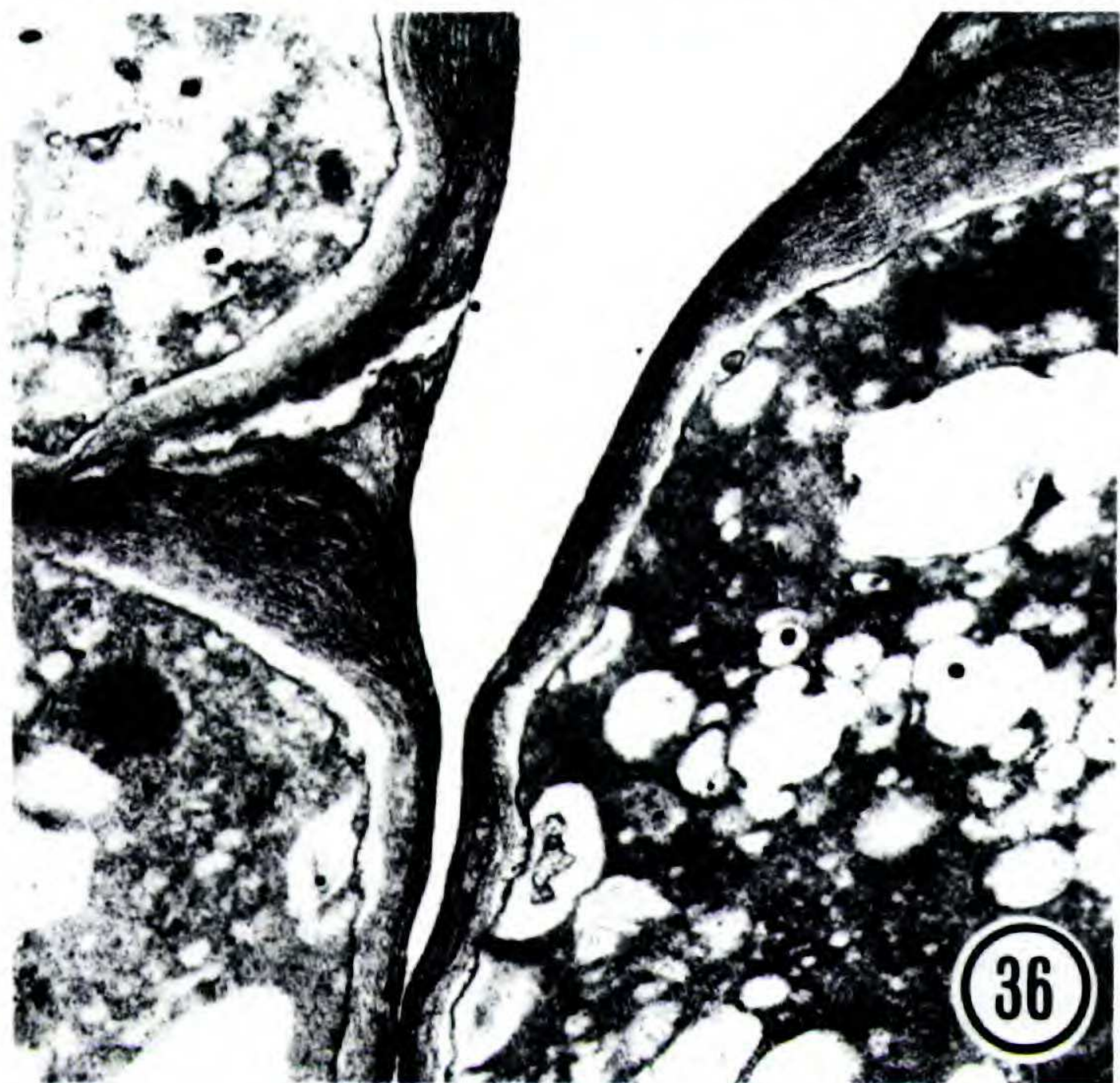
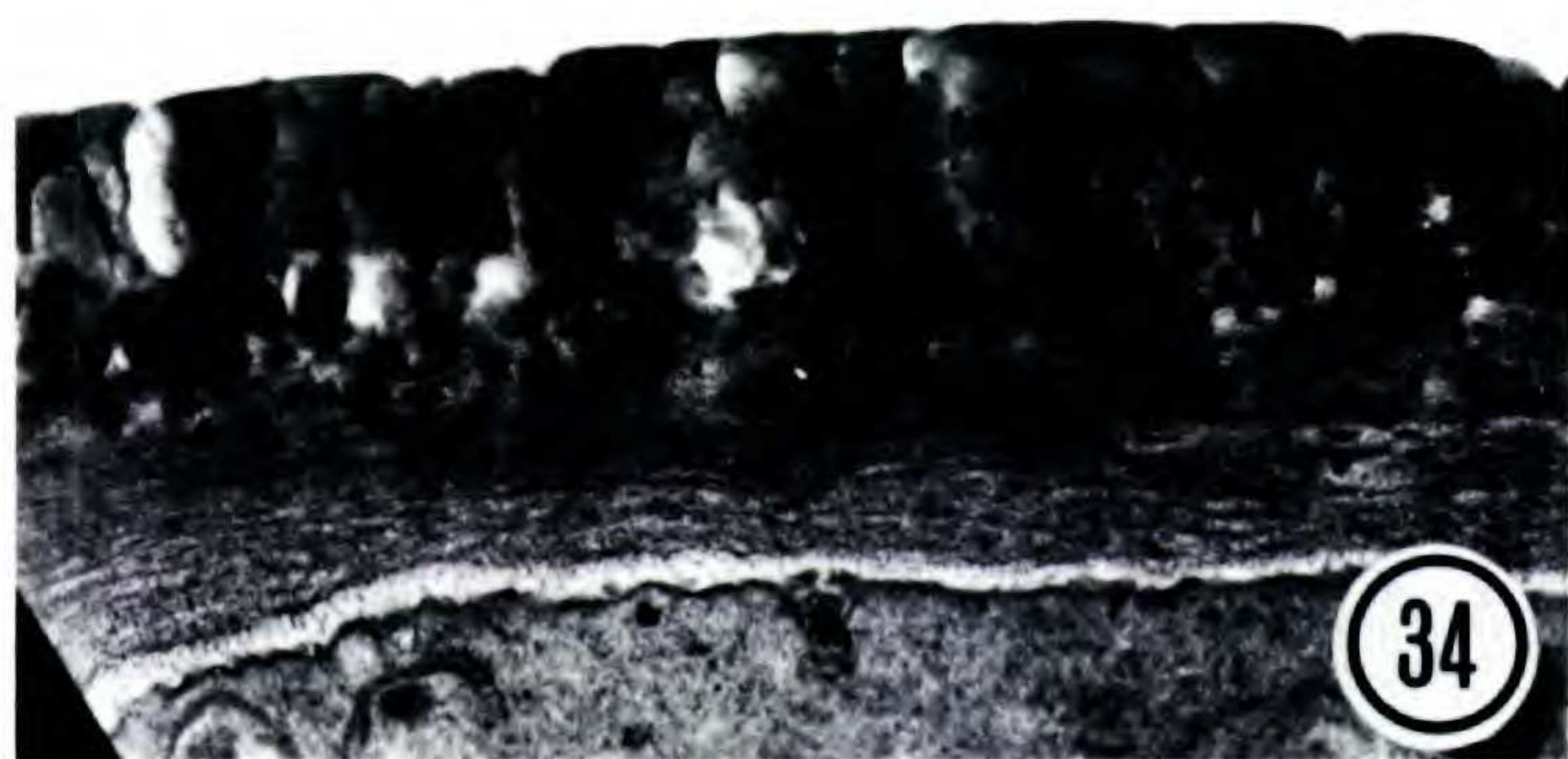
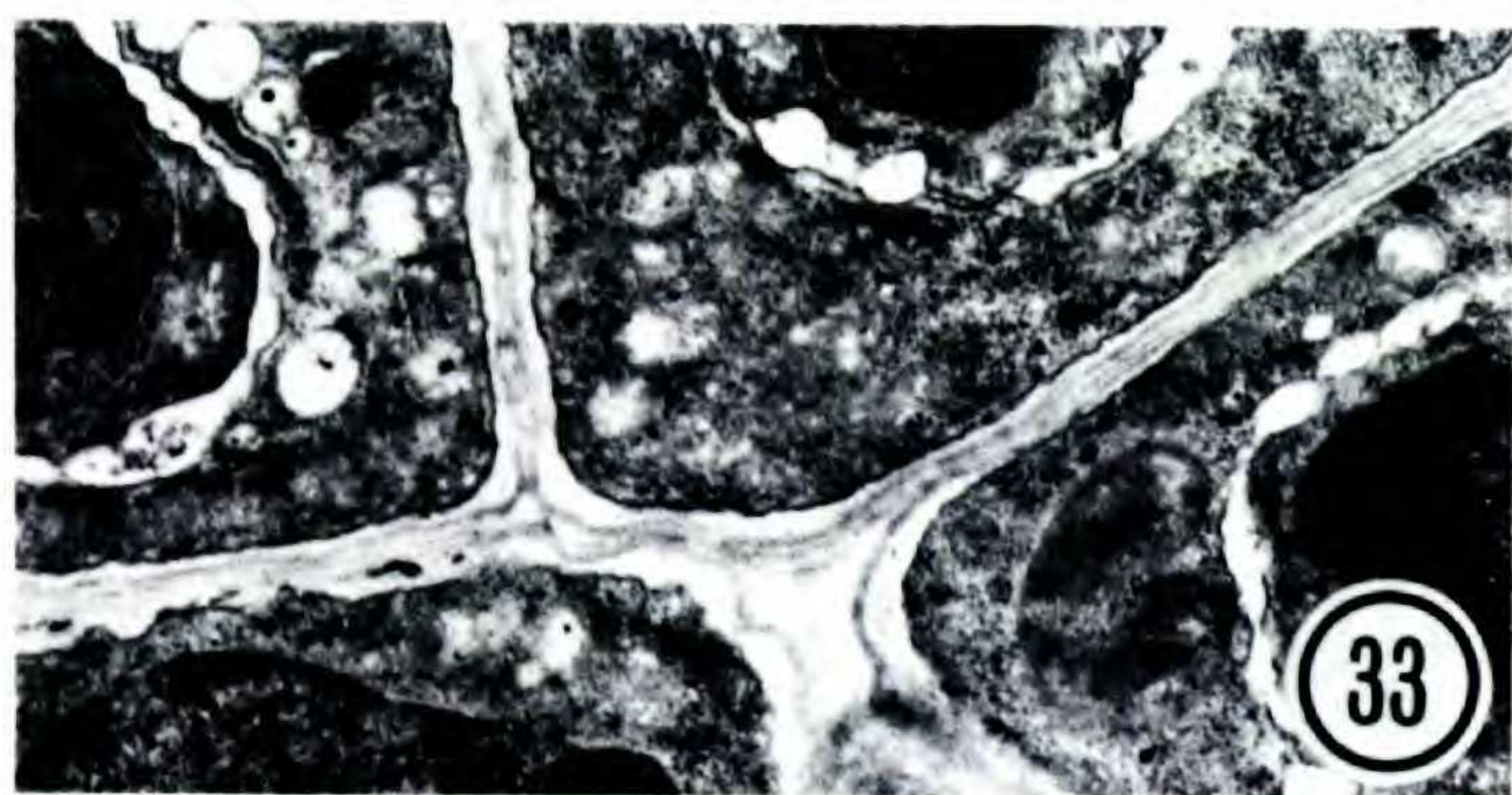
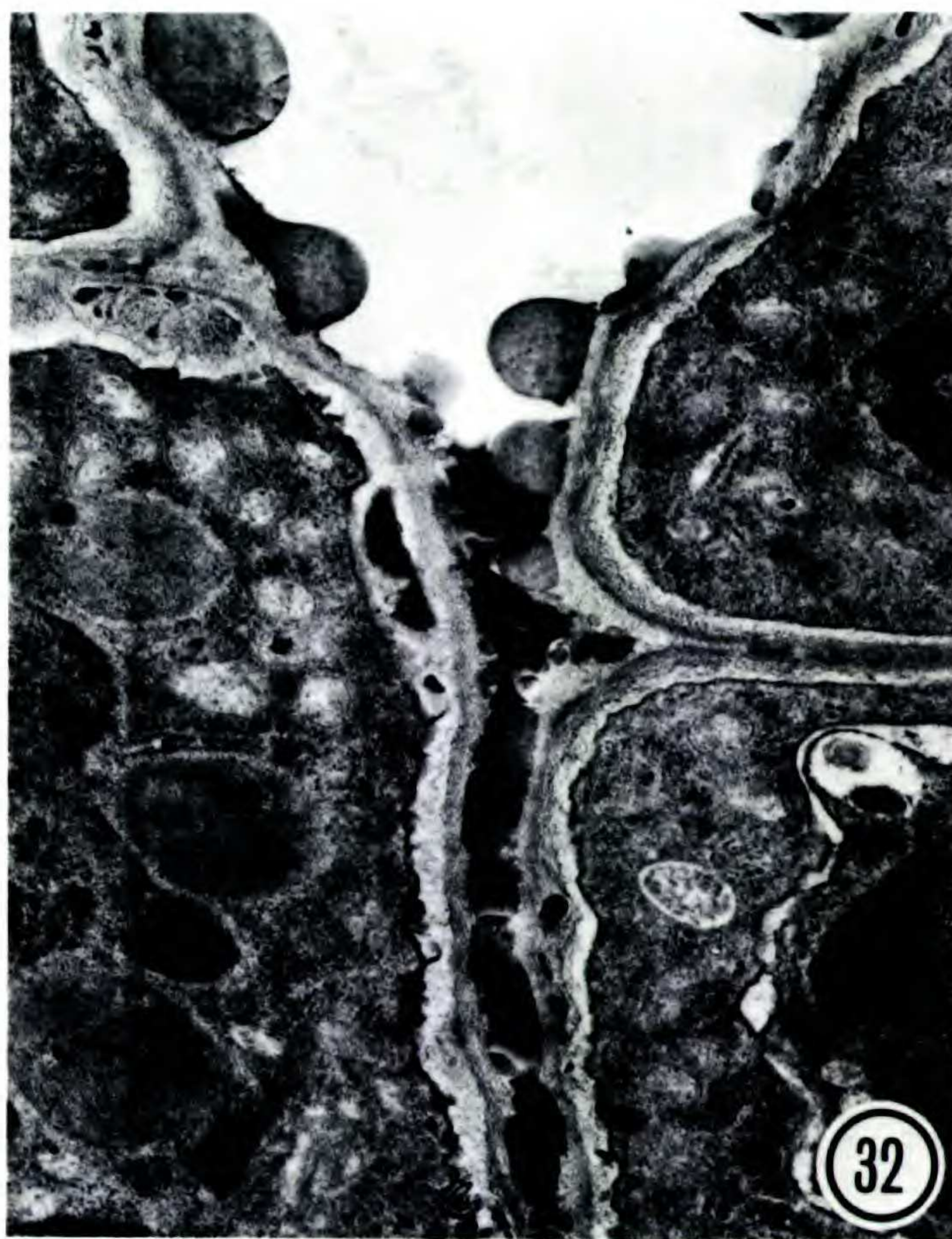
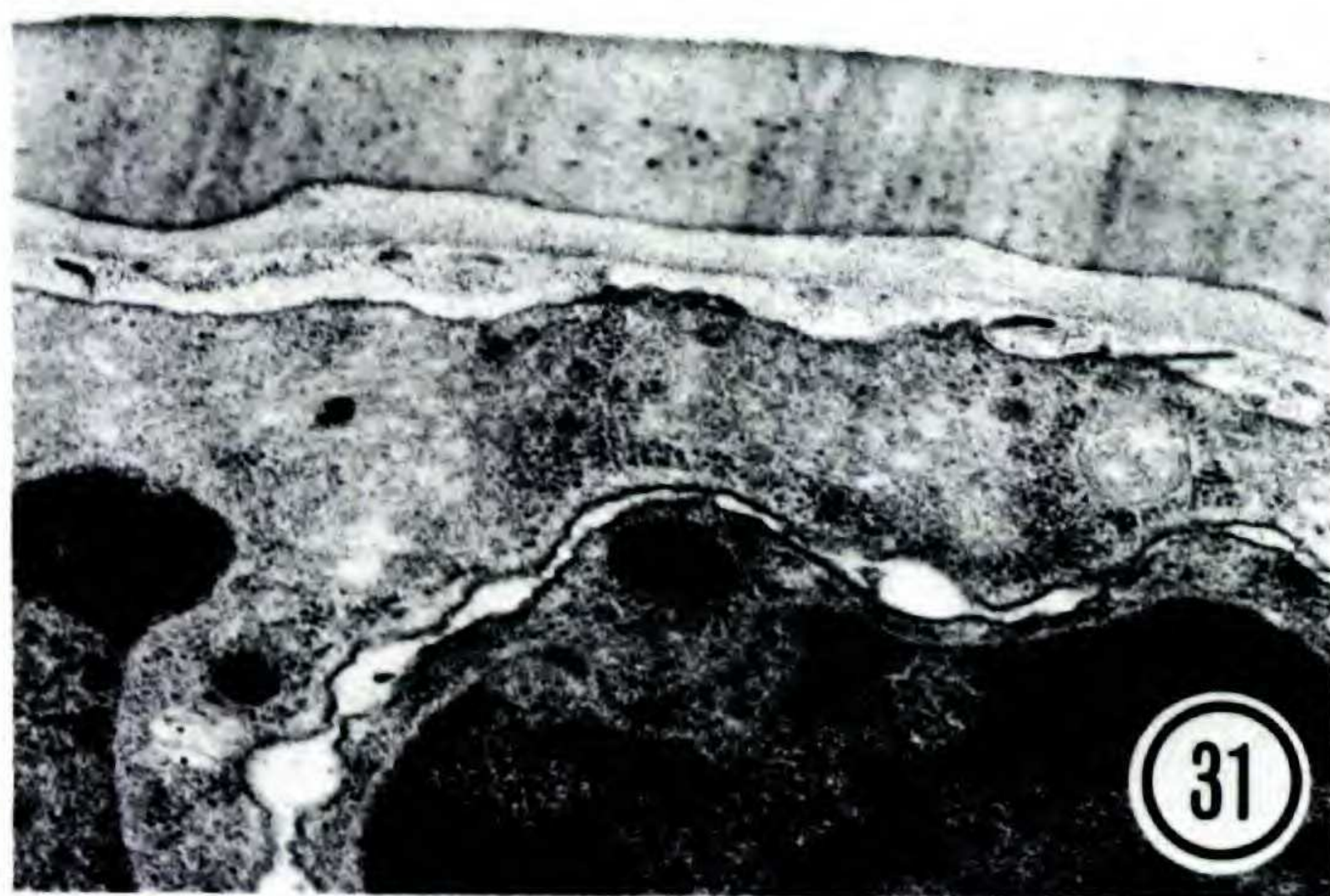
Epidendreae-Pleurothallidinae

Pollen of all four taxa investigated in this subtribe occur in tightly packed pollinia and are inaperturate.

Masdevallia caudata Lindl. Pollen grains peripheral in the pollinia have a thick homogeneous outer layer (Fig. 25). The inner portion of the exine is also homogeneous but is traversed by minute channels (Fig. 25). The inner surface of the exine is endo-rugulate and rests directly on a thin intine (Fig. 25). Pollen in the interior of the pollinium are exineless, surrounded only by the intine (Fig. 26).

Dryadella edwallii (Cogn.) Luer. The wall structure of pollen most peripheral in the pollinium consists of a thick homogeneous wall (atectate) (Fig. 27). This wall layer rests directly on a thin intine. Pollen grains in the interior of the pollinium are exineless and are surrounded by a multilayered intine (Fig. 28).

Pleurothallis janetiae Luer and *Restrepia striata* Rolfe. Both taxa have similar wall structure and will be treated together. Pollen grains peripheral in the pollinium have a thick homogeneous tectum (atectate) that rests directly on the intine (Figs. 29, 31). However, the exine becomes rugulate (the rugulae are large granular structures) on the lateral walls of the outer pollen grains (Fig. 29, 32). Pollen interior in the pollinium lack exine and are sur-



rounded only by a multilayered intine (Figs. 30, 33).

Epidendreae–Dendrobiinae

Dendrobium nobile Lindl. Pollen grains occur in tight pollinia and are inaperturate. Grains peripheral in the pollinium have a thick tectum underlain by a granular layer (Fig. 34). The tectum peters out toward the contact areas of adjacent pollen grains, where the granular layer is only present. The granular layer rests on a fibrillar intine (Fig. 34). Pollen interior in the pollinium are exineless and surrounded by a bilayered intine (Fig. 35).

D. aphyllum (Roxb.) Fischer. Pollen grains occur in loosely packed pollinia and are inaperturate. Pollen in both the peripheral and interior regions of the pollinium are exineless (Figs. 36, 37); however, pollen peripheral in the pollinium have a massive multilayered intine (Fig. 36). Pollen in the interior of the pollinium have a thin multilayered intine (Fig. 37).

Epidendreae–Bulbophyllinae

Bulbophyllum imbricatum Lindl. Pollen grains occur in tightly packed pollinia and are inaperturate. Pollen peripheral in the pollinium have a tectate-granular wall (Fig. 38), the granular layer resting directly on a thin intine. Pollen grains interior in the pollinium are exineless, surrounded only by intine (Fig. 38).

Vandaeae–Sarcanthinae

Schoenorchis fragrans (Par. & Reichb. f.) Seid. & Smit. Pollen grains occur in tightly packed pollinia and are inaperturate. Pollen peripheral in the pollinium are surrounded by a homogeneous atectate exine (Fig. 39). Pollen interior in the pollinium is exineless, and each pollen grain is surrounded by a thin bilayered intine (Fig. 39).

Ascocentrum ampullaceum (Roxb.) Schltr., *Cleisostoma racemiferum* (Lindl.) Garay, *Haraella retrocalla* (Hayata) Kudo *Luisia teres* (Thunb.). Bl.

var. *botanensis* (Fuk.) T. P. Lin, *Phalaenopsis lueddemanniana* Reichb. f., *Thrixspermum saruwatarii* (Hayata) Schltr. Pollen grains of all the species investigated occur in tightly packed pollinia. Pollen peripheral in the pollinia have a thick homogeneous, atectate wall with endo-rugulations (*Ascocentrum*, Fig. 46; *Phalaenopsis*, Fig. 40). Pollen of *Luisia*, *Thrixspermum*, *Cleisostoma*, and *Haraella* have tectate-granular exines (Figs. 42, 44, 48, 50). The exine rests on a thin (*Thrixspermum*, Fig. 42) or thick (*Phalaenopsis*, Fig. 40; *Luisia*, Fig. 45; *Ascocentrum*, Fig. 47; *Cleisostoma*, Fig. 49) multilayered intine. Pollen interior in the pollinium are exineless and are surrounded by a thick, often elaborated intine (e.g., *Thrixspermum*) (Figs. 41, 43, 45, 47, 49, 51).

Vandaeae–Zygopetalinae

Cochleanthes discolor (Lindl.) Schultes & Garay. Pollen grains occur in tightly packed pollinia. Pollen grains peripheral in the pollinium have a tectate-granular exine (Fig. 52), the granular layer resting directly on a bilayered intine (Fig. 52). Pollen grains interior in the pollinium lack exine and are surrounded only by the bilayered intine (Fig. 53).

Vandaeae–Bifrenariinae

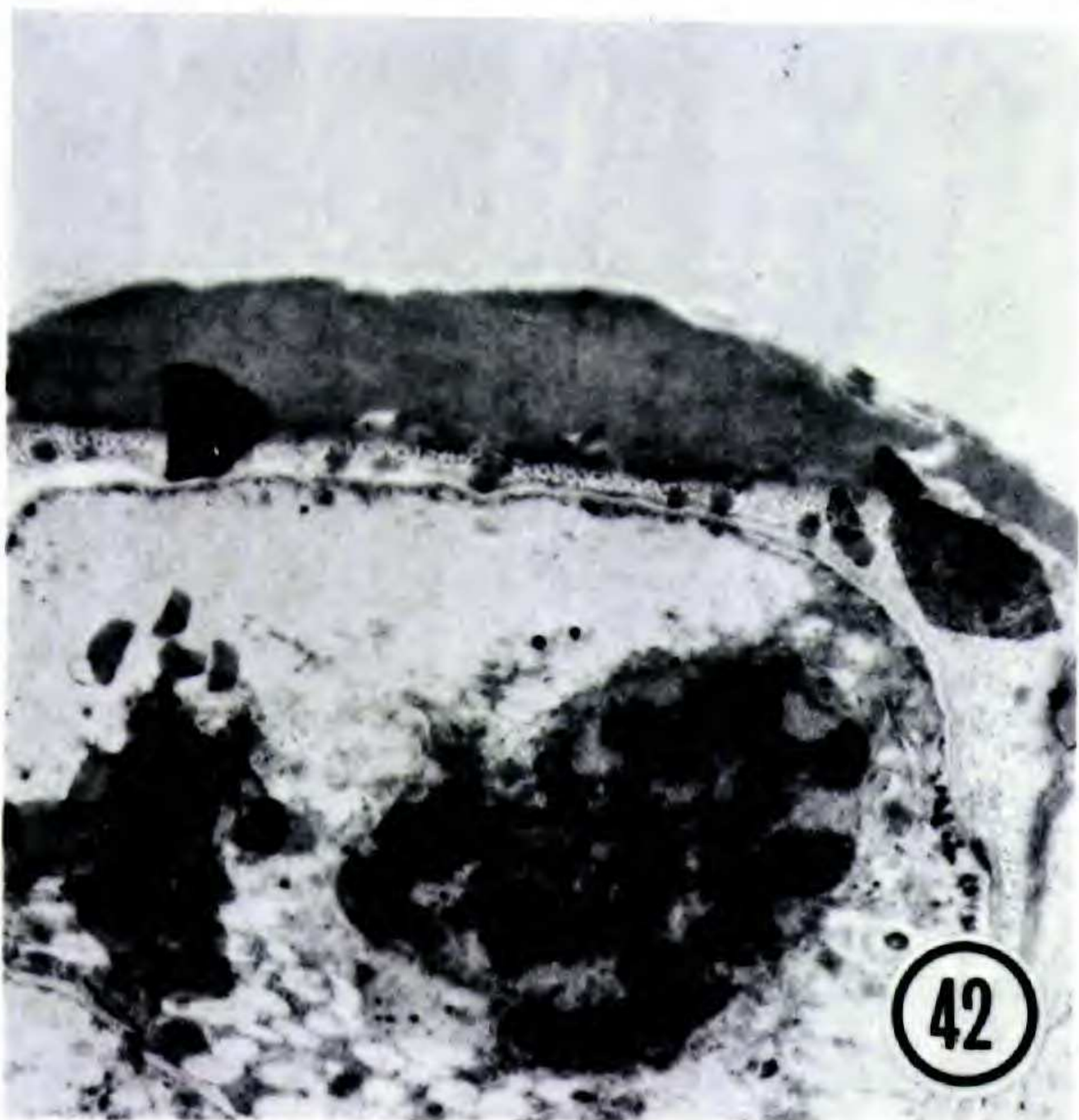
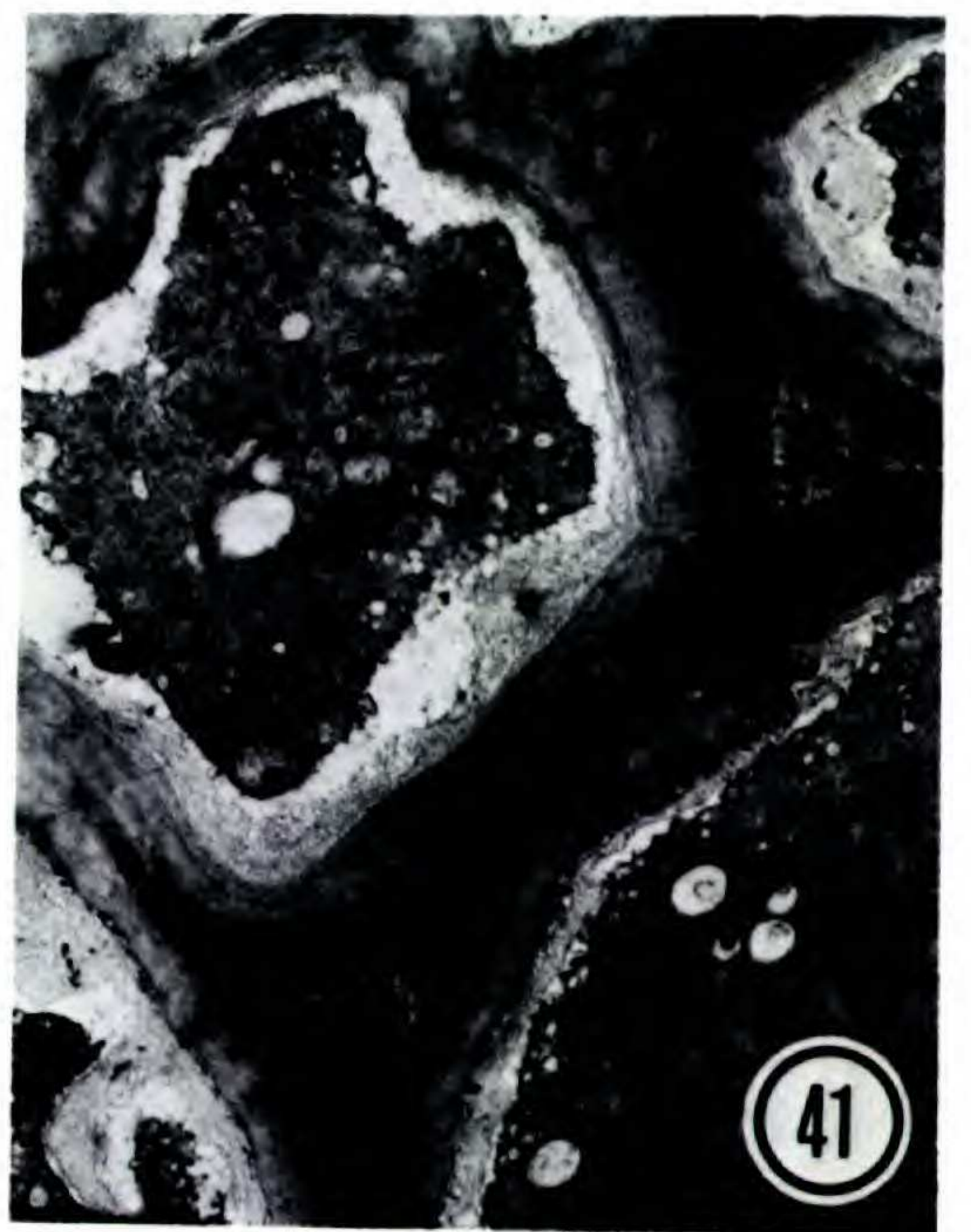
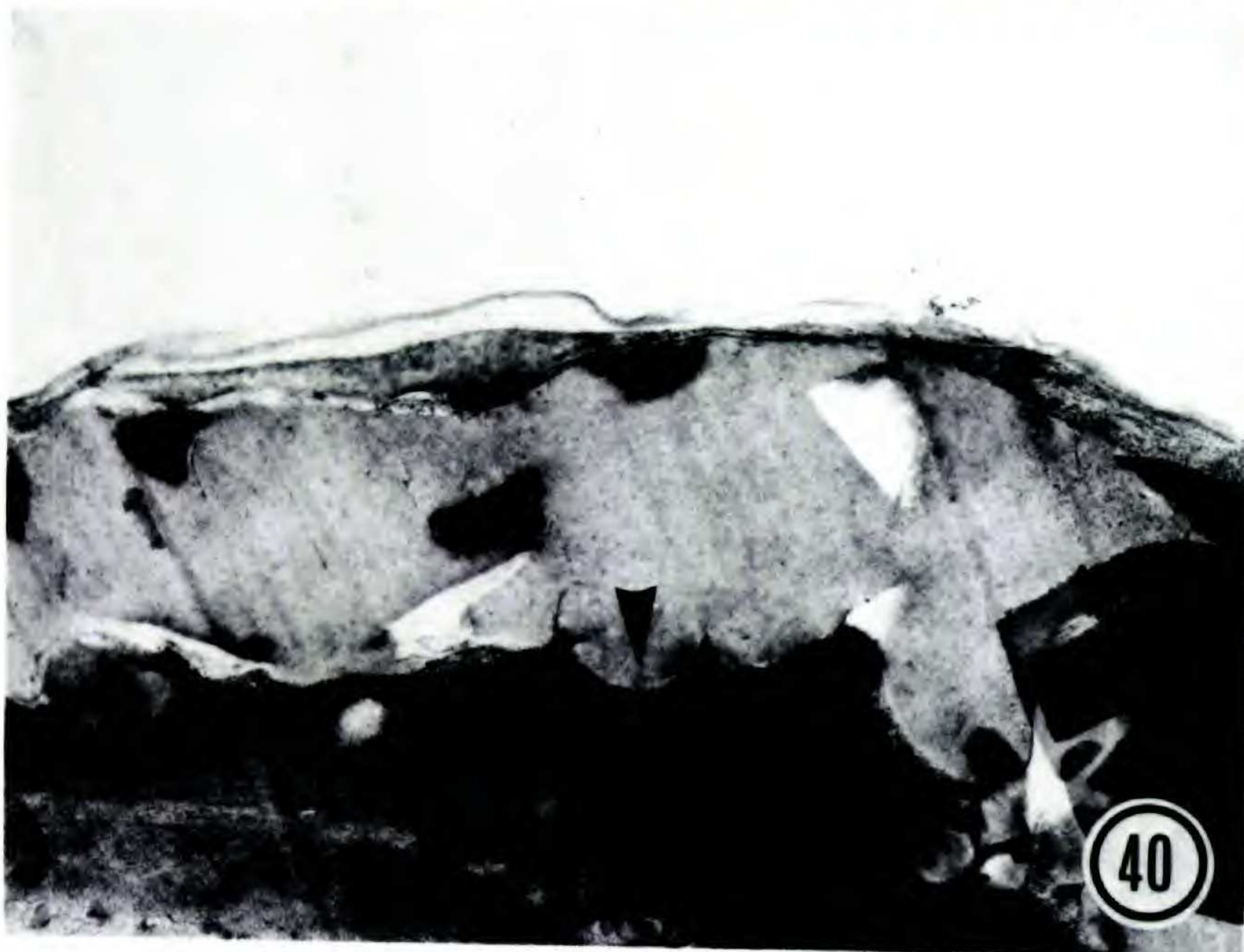
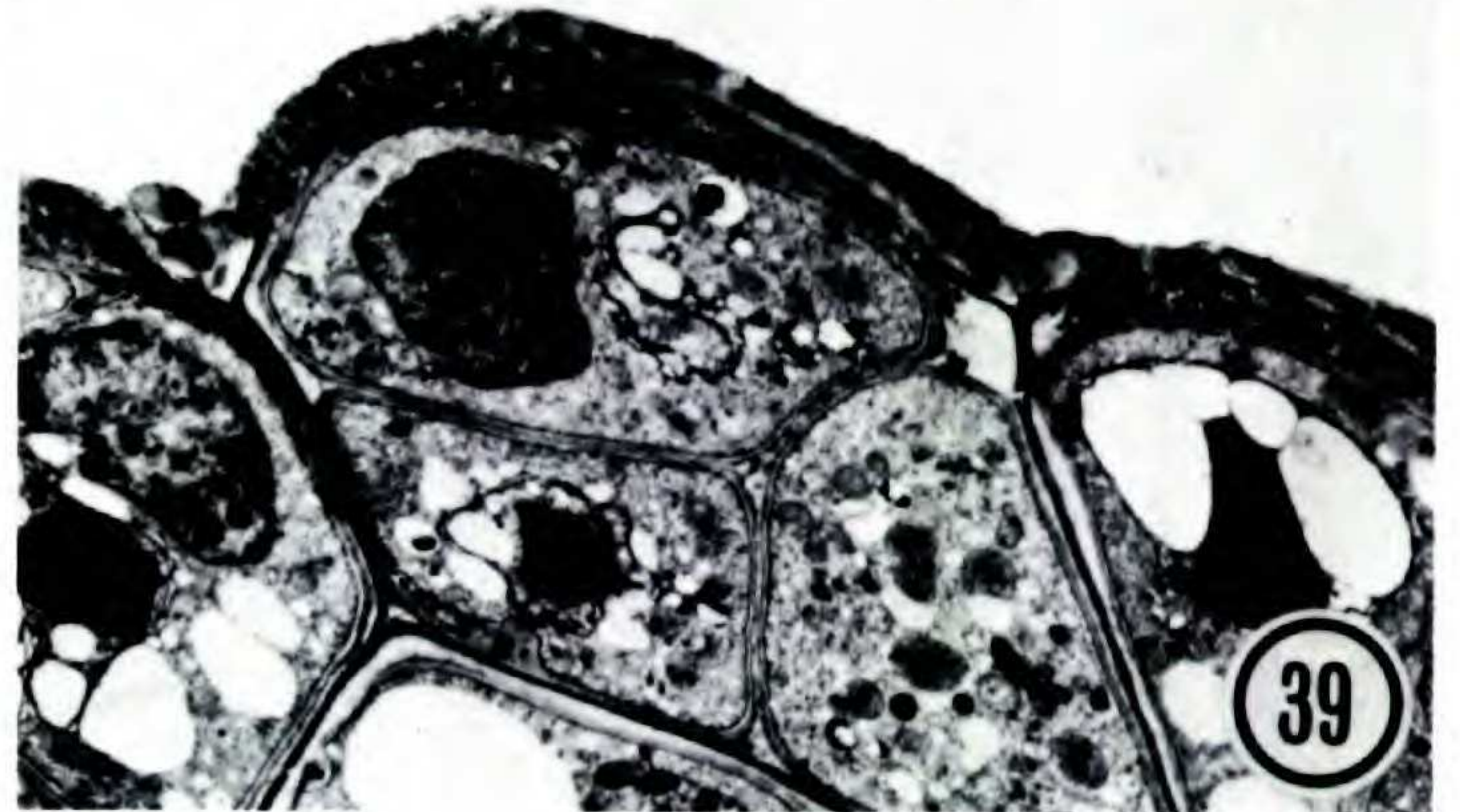
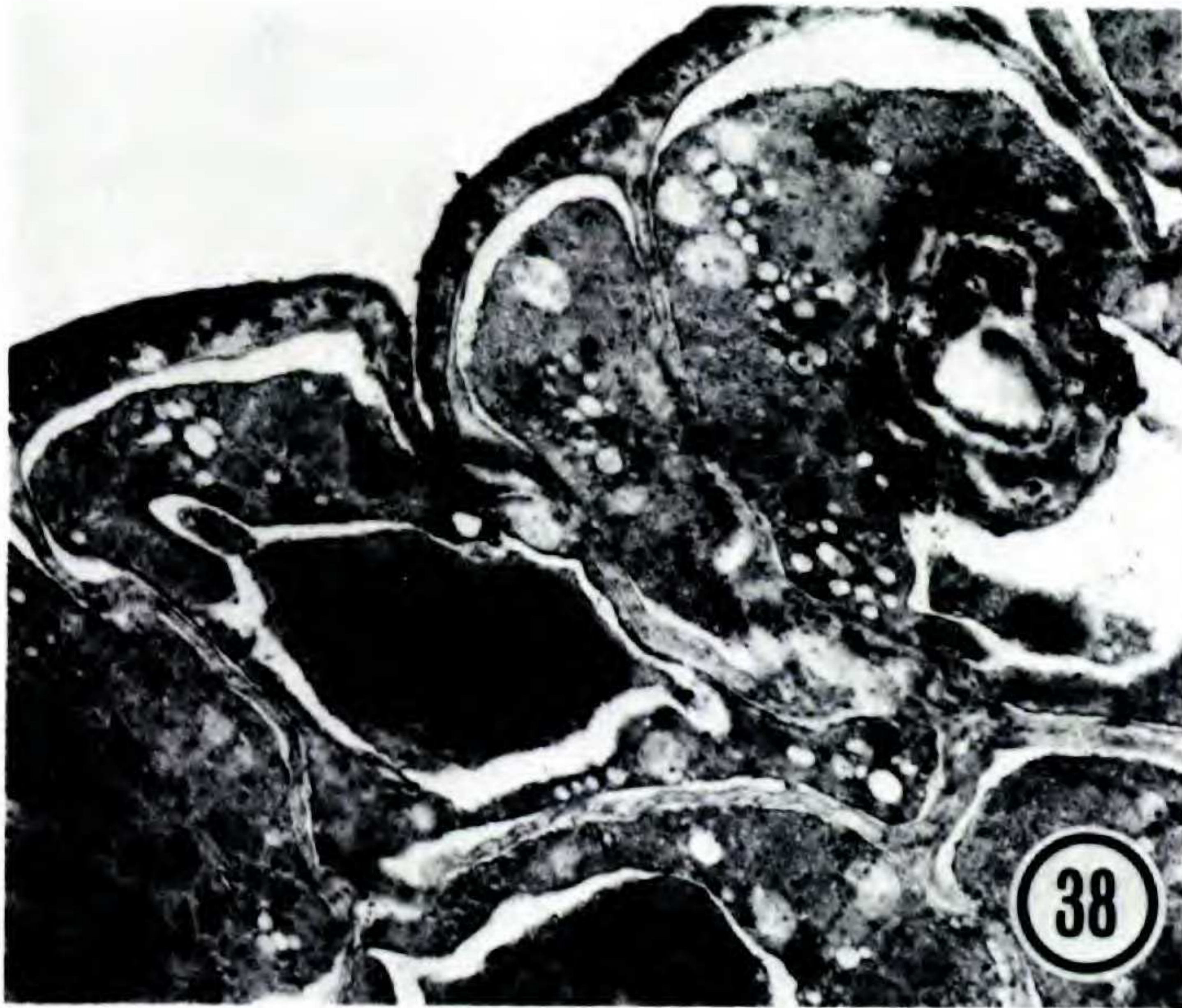
Rudolfiella aurantiaca (Lindl.) Hoehn. Pollen grains occur in tightly packed pollinia. Pollen peripheral in the pollinium have a tectate-granular exine, the granular layer resting directly on the intine (Fig. 54). The exine is perforated with minute channels (Fig. 54). Pollen in the interior of the pollinium are exineless, surrounded by a thin intine (Fig. 55); however, sporopollenin granules occasionally are embedded in the thin intine.

Vandaeae–Ornithocephalinae

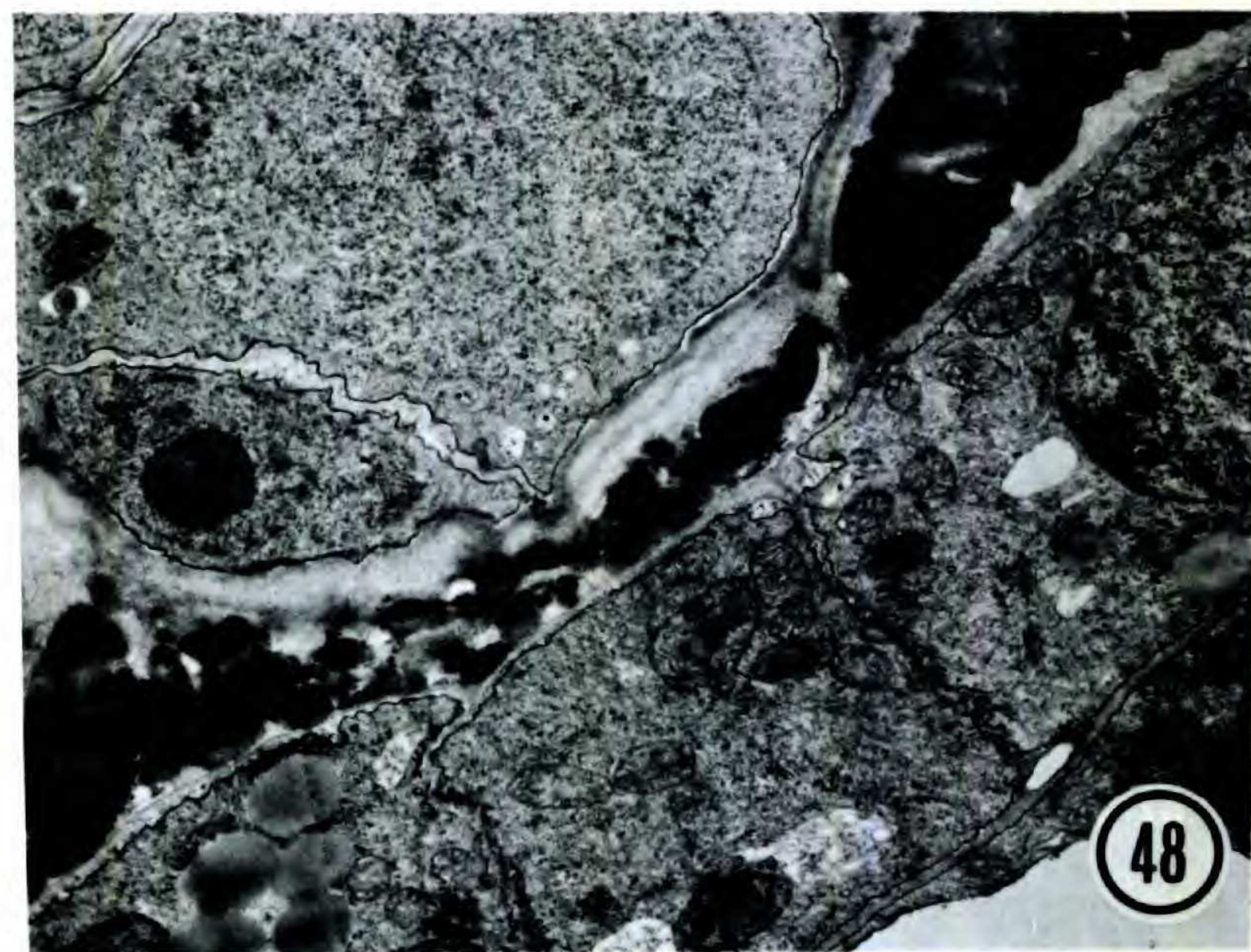
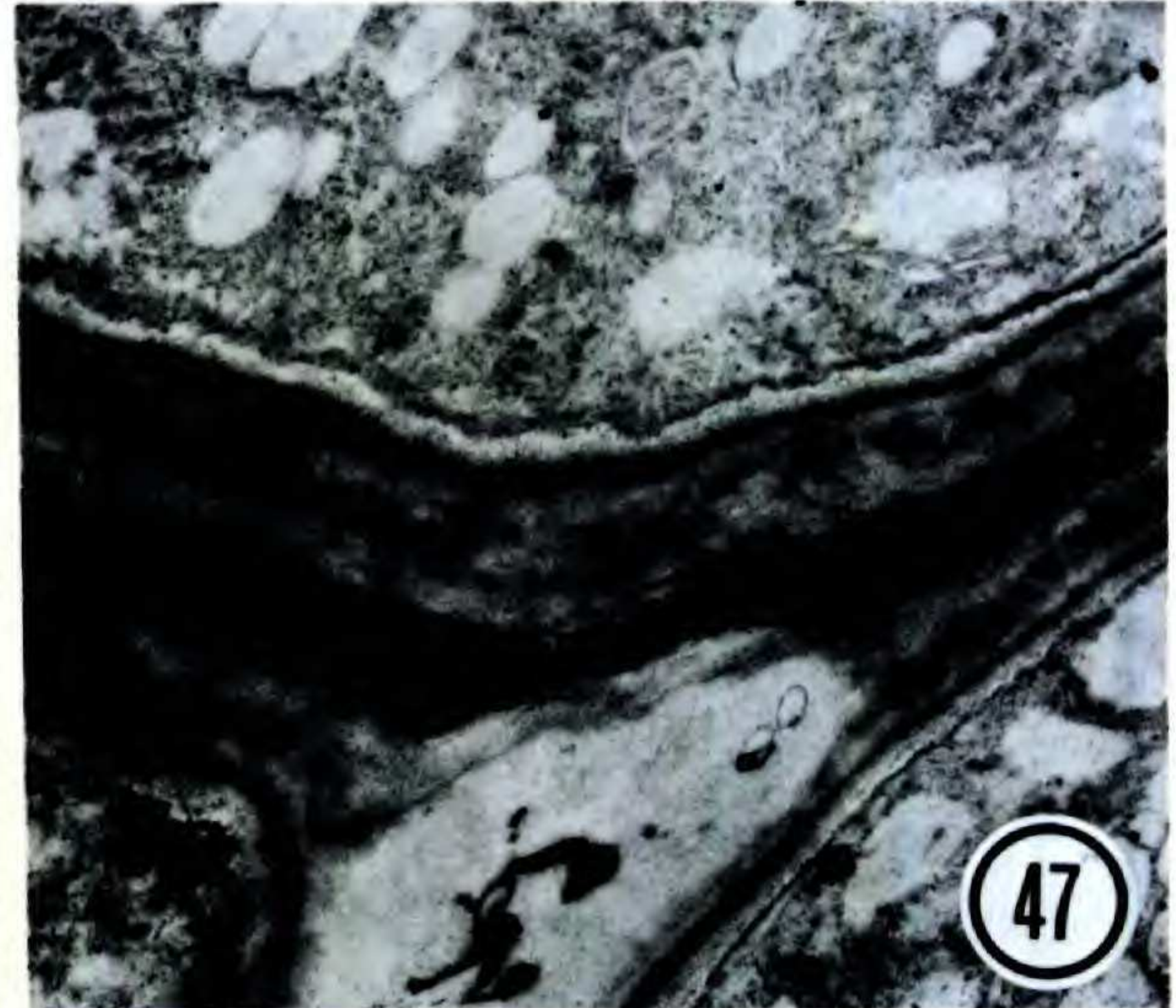
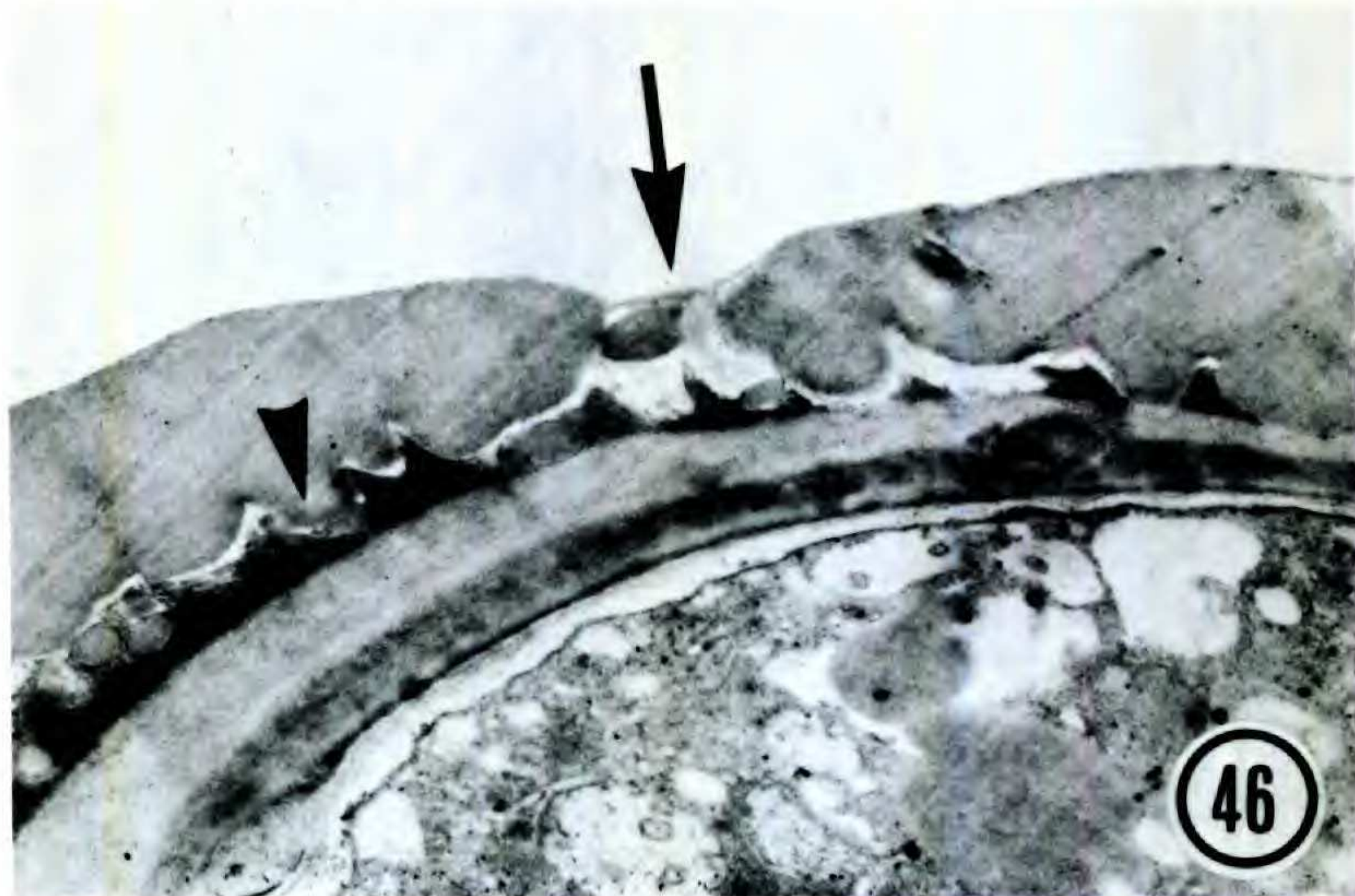
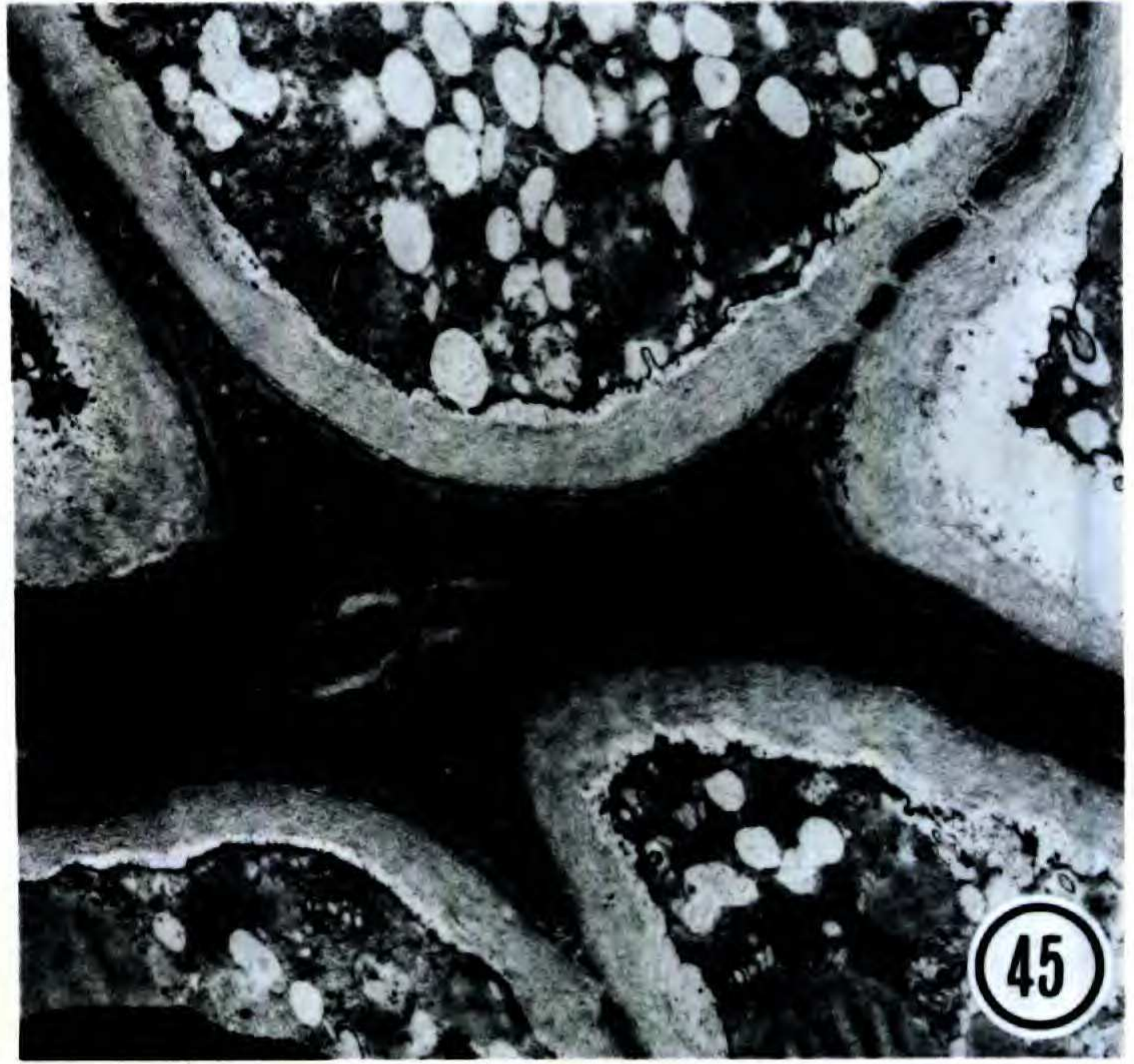
Zygostates alleniana Kraenzl. Pollen grains occur in tightly packed pollinia. Pollen peripheral in the pollinium have a tectate-granular wall structure (Fig. 56); the granular layer rests directly on a

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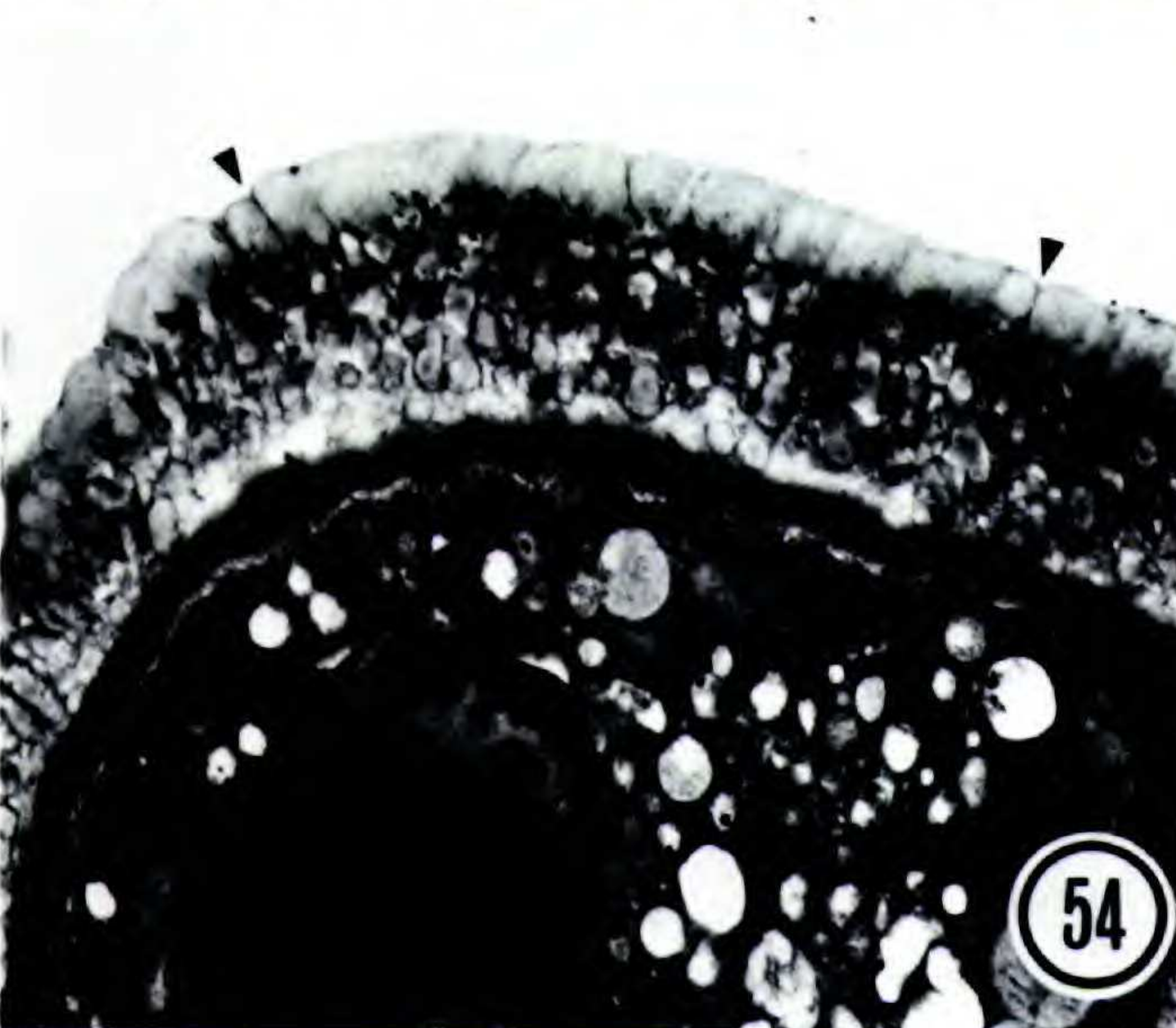
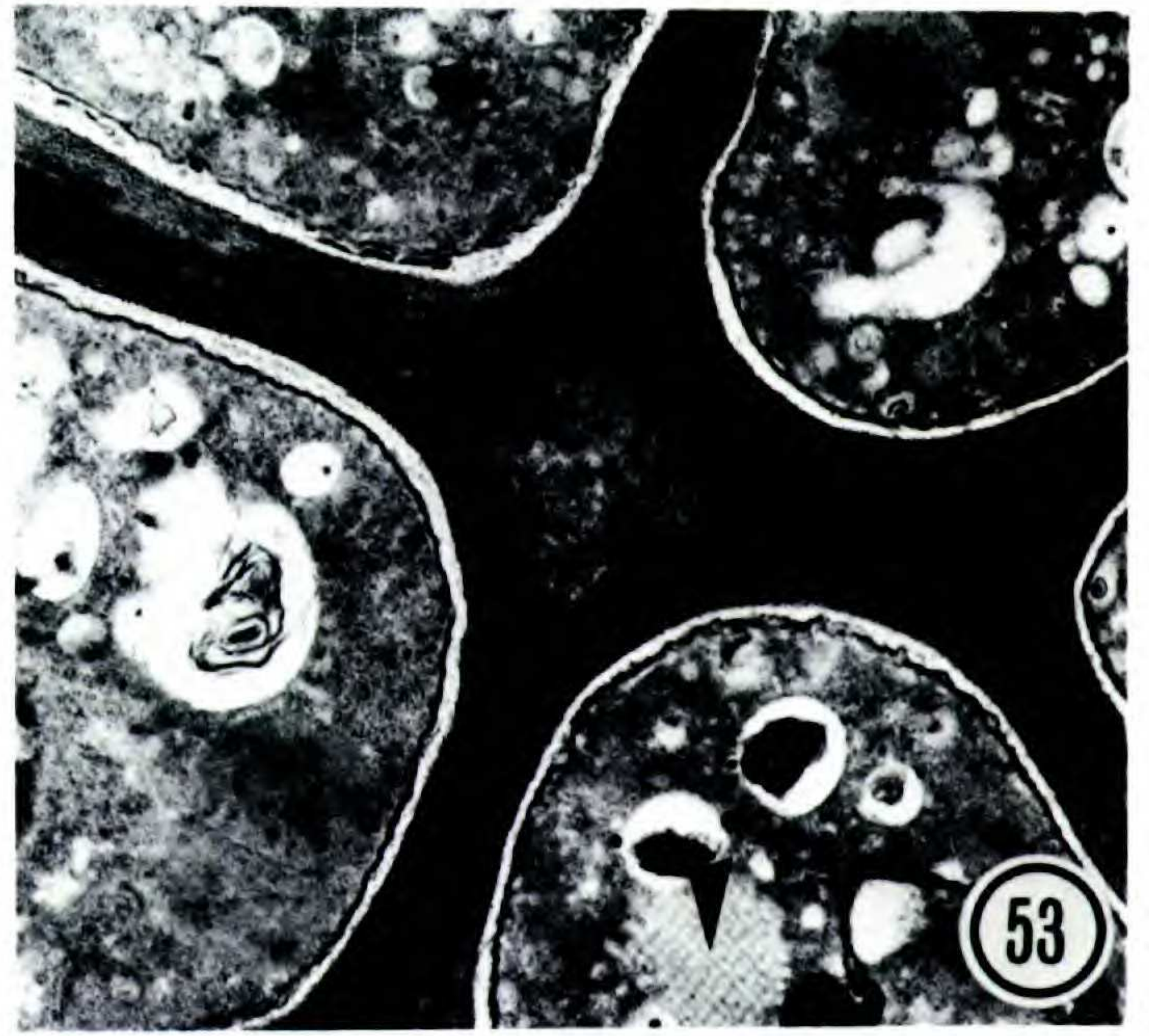
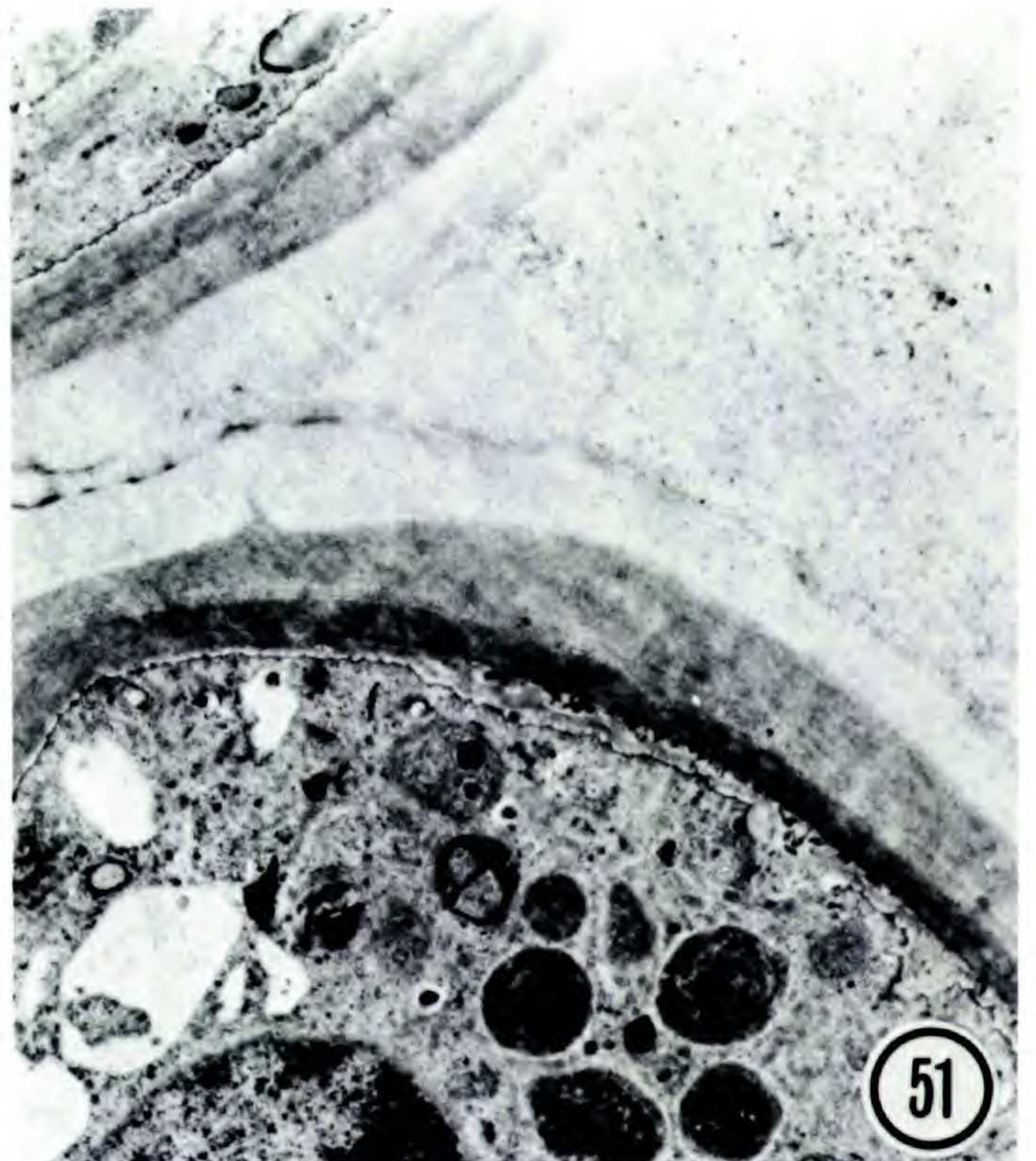
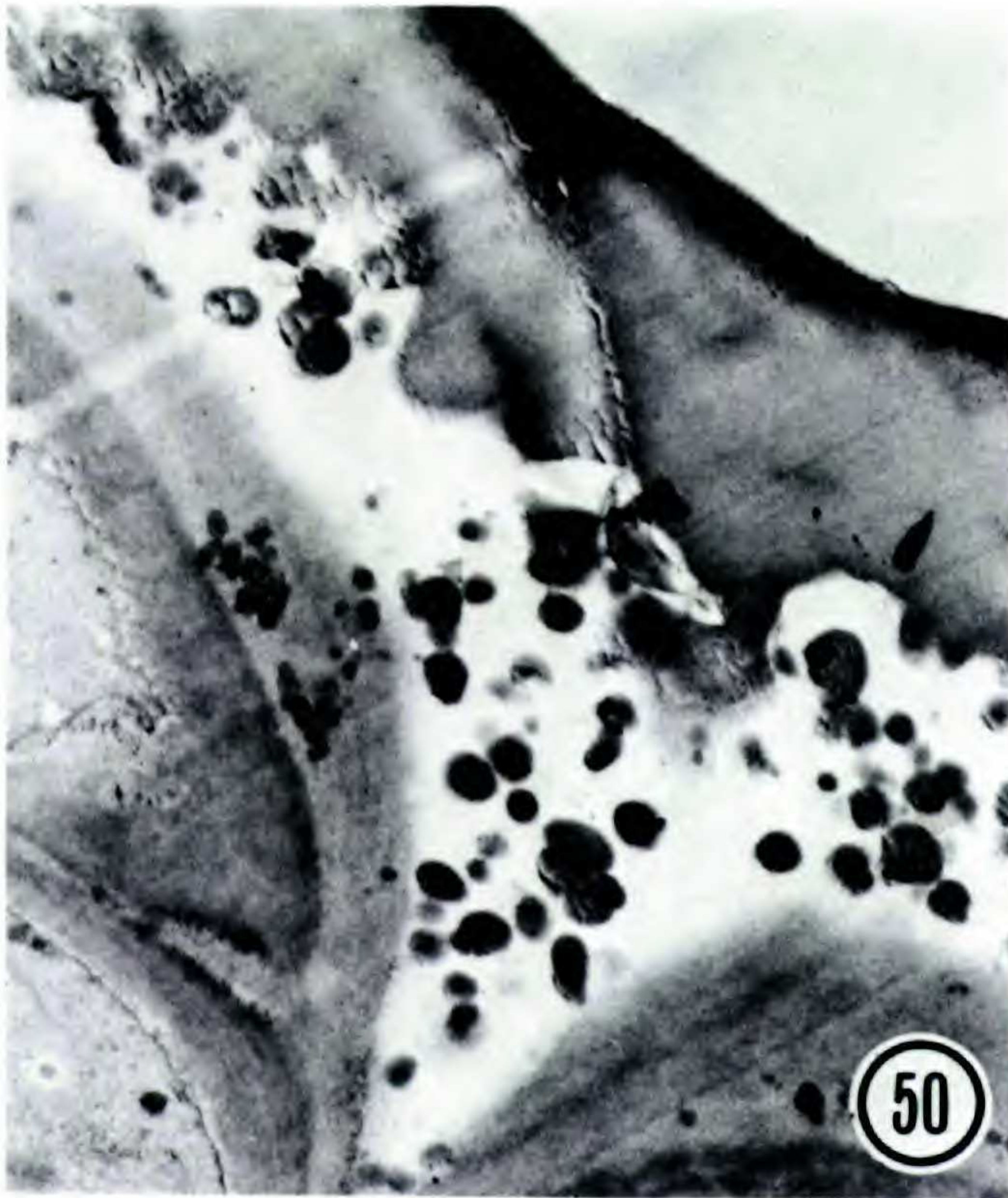
FIGURES 31–37. Pollen of the Epidendroideae.—31. Pollen peripheral in the pollinium of *Restrepia striata* showing atectate exine, $\times 20,500$.—32. *R. striata* pollen showing sporopollenin granules between adjacent pollen grains peripheral in the pollinium, $\times 16,000$.—33. *R. striata* pollen interior in the pollinium showing the thin intine, $\times 10,000$.—34. Pollen peripheral in the pollinium of *Dendrobium nobile* showing tectum and the dense granular layer that rests directly on a fibrillar intine, $\times 15,125$.—35. Pollen interior in the pollinium of *D. nobile* showing bilayered intine, $\times 6,550$.—36. Pollen peripheral in the pollinium of *D. aphyllum* showing the presence of only the fibrillar intine, $\times 5,300$.—37. Pollen interior in the pollinium of *D. aphyllum* showing loosely packed pollen and the intine, $\times 3,450$.



FIGURES 38-43. Pollen of the Epidendroideae.—38. Pollen of *Bulbophyllum imbricatum* showing outer grains with tectate-granular wall and exineless interior pollen grains, $\times 6,500$.—39. Pollen of *Schoenorchis fragrans* showing the atectate exine that is continuous around the entire pollinium and exineless interior pollen grains, $\times 3,500$.—40. Pollen peripheral in the pollinium of *Phalaenopsis lueddemanniana* showing atectate exine with endo-rugulations (arrowhead), $\times 20,500$.—41. Exineless pollen interior in the pollinium of *P. lueddemanniana* showing multilayered intine, $\times 8,200$.—42. Pollen peripheral in the pollinium of *Thrixspermum saruwatarii* showing the exine that is underlain by a scanty granular layer, $\times 6,500$.—43. Exineless pollen interior in the pollinium of *T. saruwatarii* showing elaborated intine, $\times 28,500$.



FIGURES 44-49. Pollen of the Epidendroideae.—44. Pollen peripheral in the pollinium of *Luisia teres* var. *botanensis* showing thick tectum and the thin granular infrastructural layer that rests directly on the bilayered intine, $\times 6,500$.—45. Pollen interior in the pollinium of *L. teres* var. *botanensis* showing multilayered intine. Note the small granules embedded in the dense intinous layer (arrowhead), $\times 4,150$.—46. Pollen peripheral in the pollinium of *Ascocentrum ampullaceum* showing atectate exine with endo-rugulations (arrowhead). Note the thinning of the exine; these thin areas may function as apertures (arrow), $\times 11,500$.—47. Exineless pollen interior in the pollinium of *A. ampullaceum* showing multilayered intine, $\times 10,000$.—48. Pollen of *Cleisostoma racemiferum* showing sporopolleninous wall between adjacent pollen grains, $\times 7,100$.—49. Exineless pollen inferior in the pollinium of *C. racemiferum*, $\times 3,500$.



bilayered intine (Fig. 56). In the tectum are urn-shaped structures of unknown function (this feature is not known to occur in any other pollen, gymnosperm, or angiosperm) (Fig. 56). Pollen grains in the interior of the pollinium are exineless and are surrounded by a bilayered intine (Fig. 57).

Cymbidieae

Cymbidium aloifolium (L.) Sw. Pollen grains occur in tightly packed pollinia. Pollen peripheral in the pollinium have a tectate-granular wall structure (Fig. 58), the granular layer resting directly on the intine (Fig. 58). The tectum is traversed by minute channels (Fig. 58). Pollen interior in the pollinium are loosely attached and exineless; the intine is the only wall layer present (Fig. 59). However, sporopollenin granules are occasionally embedded in the intine (Fig. 59).

Cymbidieae–Oncidiinae

Trichophilia marginata Henfr. Pollen grains occur in tightly packed pollinia. Pollen peripheral in the pollinium have a tectate-granular wall structure (Fig. 60). The granular layer rests on a thin intine (Fig. 60). Pollen interior in the pollinium are exineless and surrounded by a multilayered intine (Fig. 61); however, sporopollenin granules often occur in the contact areas between adjacent pollen grains (Fig. 61).

DISCUSSION

Pollen grains from each of the five orchid subfamilies were studied ultrastructurally. Although the number of species studied is insubstantial in relation to the size of the family, the available data, when considered in the context of other morphological features, provide insight into the evolutionary trends of aperture and wall structure of the Orchidaceae.

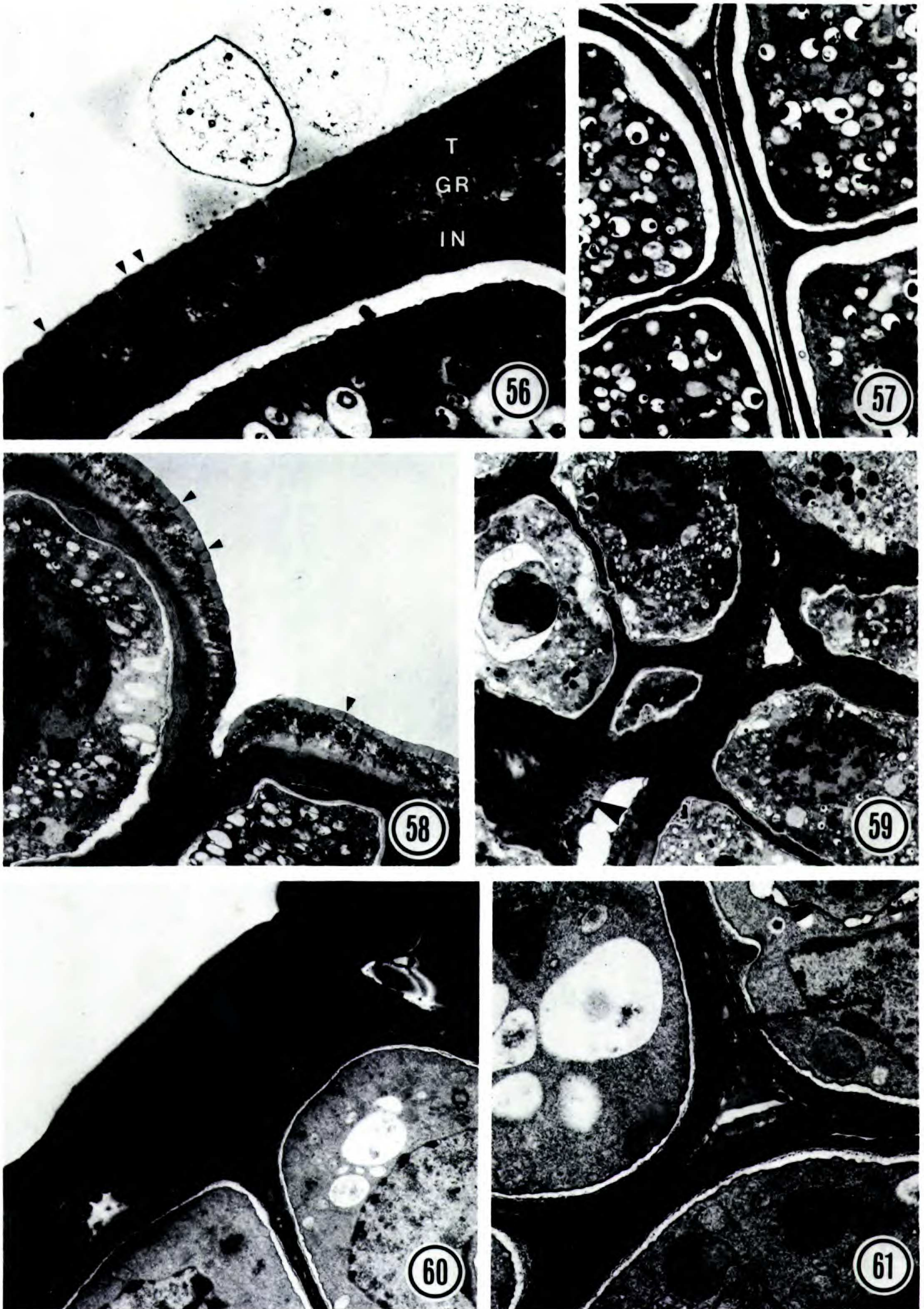
Burns-Balogh (1983) has proposed evolutionary trends for exine structure of orchid pollen. She considered pollen of the Apostasioideae most prim-

itive. This is based on the pollen characters associated with unspecialized reproductive organs of the subfamily. The pollen wall of *Apostasia* is tectate-perforate-columellate with a well-developed footlayer. Burns-Balogh (1983) recognized two major lines diverging from the Apostasioideae. One line culminates in the tectate-imperforate-granular wall of the Cypripedioideae via a tectate-imperforate-columellate wall. The other line progresses to the tectate-perforate-columellate type, eventually giving rise to tectate-granular wall types of the Orchidoideae and Epidendroideae with the loss of the footlayer. In all of these types Burns-Balogh (1983) recognized the common occurrence of endexine. Endexine does not usually occur in monocots (Zavada, 1983), and this wall layer has not been unequivocally demonstrated in orchids: the footlayer or infrastructural layer in all cases rests directly on a fibrillar intine. Although Burns-Balogh's (1983) treatment of evolutionary trends of wall structure in orchids may be reasonable, the small sample size does not permit placing the large subfamilies in an evolutionary sequence based on pollen alone. In addition, some workers have suggested that the large subfamily Epidendroideae may be polyphyletic (Dressler, 1983). The data in the present contribution will be treated in terms of major evolutionary trends of pollen wall structure within the Orchidaceae (not necessarily representing cladistic relationships). This will be subject to revision and refinement as data accumulate on pollen ultrastructure in this family.

Three major trends are evident in the Orchidaceae. The first and most obvious is toward adherence of pollen grains into polyads (pollinia). This trend is best developed in the Orchidaceae and does not characterize many other monocot groups. However, polyads occur in the dicots (e.g., Asclepiadaceae, Fabaceae). In dicots with polyads, there is observed a pollen wall ultrastructure comparable to that of the orchids, i.e., a shift from the tectate-columellate wall structure in the taxa considered least specialized to tectate-granular or atectate in the taxa considered derived (Dicko-Zafimahova &

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FIGURES 50–55. Pollen of the Epidendroideae.—50. Pollen peripheral in the pollinium of *Haraella retrocalla* showing the thick continuous tectum and scanty granular infrastructure, $\times 7,850$.—51. Exineless pollen interior in the pollinium of *H. retrocalla* showing bilayered intine, $\times 7,850$.—52. Peripheral pollen in the pollinium of *Cochleanthes discolor* showing tectum, granular infrastructure, and bilayered intine, $\times 10,000$.—53. Exineless pollen interior in the pollinium of *C. discolor* showing intine. Note paracrystalline material of unknown composition (arrowhead), $\times 10,000$.—54. Pollen peripheral in the pollinium of *Rudolfiella aurantiaca* showing tectum (with microchannels, arrowheads), granular infrastructure, and thin intine, $\times 10,000$.—55. Exineless pollen interior in the pollinium of *R. aurantiaca* showing bilayered intine, $\times 10,000$.



FIGURES 56-61. Pollen of the Epidendroideae. —56. Pollen peripheral in the pollinium of *Zygostates alleniana* showing tectum (T) with the unusual urn-shaped structures of unknown function (arrowheads), granular infrastructure (GR), and intine (IN), $\times 11,500$. —57. Exineless pollen interior in the pollinium of *Z. alleniana* showing intine,

Audran, 1981). This raises the possibility that pollen wall structure in taxa with polyads may be more related to the spatial configuration of the pollen and development of the sporopollenin wall around the pollinia than a reflection of the evolutionary level attained by a particular taxon.

Within the Orchidaceae the Apostasioideae have monads, but polyads and/or mealy pollinia occur in the Cypripedioideae and Spiranthoideae. The Orchidoideae and Epidendroideae have tightly packed, waxy pollinia (Dressler, 1981, 1983).

The second trend is toward a well-defined sulcus in the Apostasioideae, an ill-defined ulcerate pore in some Cypripedioideae and Spiranthoideae, and the inaperturate condition in the Orchidoideae and Epidendroideae (Zavada, 1983). The monosulcate condition is found in the least specialized subfamily, Apostasioideae. In the more derived subfamilies (Cypripedioideae, Spiranthoideae) the ulcerate condition is known, and in the most derived taxa (Epidendroideae) the inaperturate condition is prevalent. These trends in apertures parallel those observed in the other monocot groups, and in general, are characteristic of pollen of the Alismatidae and Zingiberidae (monosulcate to inaperturate condition). The Commelinidae exhibit a parallel trend in the reduction of the sulcus to an ill-defined pore, but here culminate in the development of a well-defined graminoid pore. The inaperturate condition is not observed in this group (Zavada, 1983).

The third major trend concerns exine structure and stratification. The occurrence of the tectate-columellate wall structure and monosulcate aperture in the Apostasioideae is typical of numerous monocot families including those generally considered primitive (Zavada, 1983 and references therein). There are a number of species in the more derived subfamilies that exhibit primitive wall structure type (tectate-columellate; Cypripedioideae, Spiranthoideae, Orchidoideae, and a few Epidendroideae). However, the primitive wall structure type often occurs with the more derived apertural types and pollen units. The combination of primitive and derived features in the few taxa investigated does not reveal the phylogenetic relationships among the Apostasioideae and these other subfam-

ilies. In addition, the derived wall structure types (tectate-granular) also occur in the derived taxa (e.g., Orchidoideae and Epidendroideae).

The Vandaeae consistently exhibit the tectate-granular and atectate wall types, no footlayer, inaperturate pollen, and compact pollinia, suggesting this is the most palynologically derived group of the family.

The orchids parallel many of the palynological evolutionary trends proposed for the other monocots (Zavada, 1983). However, a few features are unique to the monocots and the Orchidaceae in general. The first is loss of the sulcus via the ulcerate-porate condition. This is a major monocot trend, but is relatively rare in dicots. This trend, as mentioned above, occurs in the Alismatidae, Zingiberidae, and in part, the Commelinidae. The second is the occurrence of the tectate-columellate wall structure in monocots considered primitive and the least specialized orchid subfamily, Apostasioideae. The more advanced taxa have the tectate-granular, atectate, or exineless pollen. This is also a major monocot trend and is restricted to only a few dicot groups. Although some derived dicots have a granular or atectate wall structure, these wall structure types are thought to be primitive in some dicots (Walker, 1974). Concomitant with the loss of the tectum, infrastructure, and footlayer, there is elaboration of the intine in many monocots including the Orchidaceae. The occurrence of the elaborated intine is often found in monocot taxa that have specialized pollination mechanisms (e.g., Cannaceae, Kress & Stone, 1982). However, the morphological diversity and significance of the intine to pollination mechanisms and reproductive biology need to be investigated further.

Further pollen studies of orchids will undoubtedly bring many surprises. It is significant that orchids exhibit a wide range of pollen types that occur in the most primitive to the most derived monocots. Further detailed palynological studies of orchids and closely related families will be important to our understanding of pollen evolution, not only in the Orchidaceae, but in the monocots in general.

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×5,300.—58. Peripheral pollen in the pollinium of *Cymbidium aloifolium* showing microchannelled tectum (arrowheads), granular infrastructure, and thick intine, ×5,300.—59. Pollen interior in the pollinium of *C. aloifolium* showing intine with minute sporopollenin granules (arrowhead), ×3,450.—60. Pollen peripheral in the pollinium of *Trichophila marginata* showing thick tectum and massive granular infrastructure that rests on a thin intine, ×6,500.—61. Pollen interior in the pollinium of *T. marginata* showing sporopollenin granular layer (arrow) and dense intine, ×11,500.

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APPENDIX I. Specimens investigated in this study.

- Acanthephippium sylhetense* Lindl., Christenson 381 CONN, India
- Ascocentrum ampullaceum* (Roxb.) Schltr., Christenson 404 CONN, Thailand
- Bletilla striata* (Thunb.) Reichb. f., Christenson 374 CONN, Japan
- Bulbophyllum imbricatum* Lindl., Christenson 426 CONN, Africa
- Cattleya skinneri* Batem., Christenson 393 CONN, Costa Rica
- Cleisostoma racemiferum* (Lindl.) Garay, Christenson 365 CONN, India
- Cochleanthes discolor* (Lindl.) Schultes & Garay, Christenson 436 CONN, Costa Rica
- Coelogyne nitida* Lindl., Christenson 1017 CONN, India
- Cymbidium aloifolium* (L.) Sw., Christenson 384 CONN, ex hort.
- Dendrobium aphyllum* (Roxb.) Fischer, Christenson 837 CONN, India
- Dendrobium nobile* Lindl., Christenson 387 CONN, India
- Dryadella edwallii* (Cogn.) Luer, Christenson 1015 CONN, Brazil
- Encyclia cochleata* (L.) Lemee, Christenson 1016 CONN, ex hort.
- Epidendrum anceps* Jacq., Christenson 383 CONN, Florida
- Haraella retrocalla* (Hayata) Kudo, Christenson 1022 CONN, Taiwan
- Laelia autumnalis* Lindl., Christenson 366 CONN, Mexico
- Luisia teres* (Thunb.) Bl. var. *botanensis* (Fuk.) T. P. Lin, Christenson 433 CONN, Taiwan
- Malaxis latifolia* Sm., Christenson 400 CONN, ex hort.
- Malaxis paludosa* Sw., Reeves, 18 Aug. 1984 MRD, Minnesota
- Malaxis unifolia* Michx. Reeves, sin., 19 Aug. 1984 MRD, Minnesota
- Masdevallia caudata* Lindl., Christenson 379 CONN, Colombia
- Phalaenopsis lueddemanniana* Reichb. f., Christenson 391 CONN, Philippines
- Pleurothallis janetiae* Luer, Christenson 1014 CONN, Costa Rica
- Restrepia striata* Rolfe, Christenson 1018 CONN, ex hort.
- Rudolfiella aurantiaca* (Lindl.) Hoehne, Christenson 386 CONN, Brazil

Sacoila lanceolata (Aubl.) Garay, Christenson 1013
CONN, Florida

Schoenorchis fragrans (Par. & Reichb. f.) Seid. & Smit.,
Christenson 1019 CONN, India

Thrixspermum saruwatarii (Hayata) Schltr., Christenson
1021 CONN, Taiwan

Tricophilia marginata Henfr., Christenson 380 CONN,
Guatemala

Zygostates alleniana Kraenzl., Christenson 1020 CONN,
Brazil